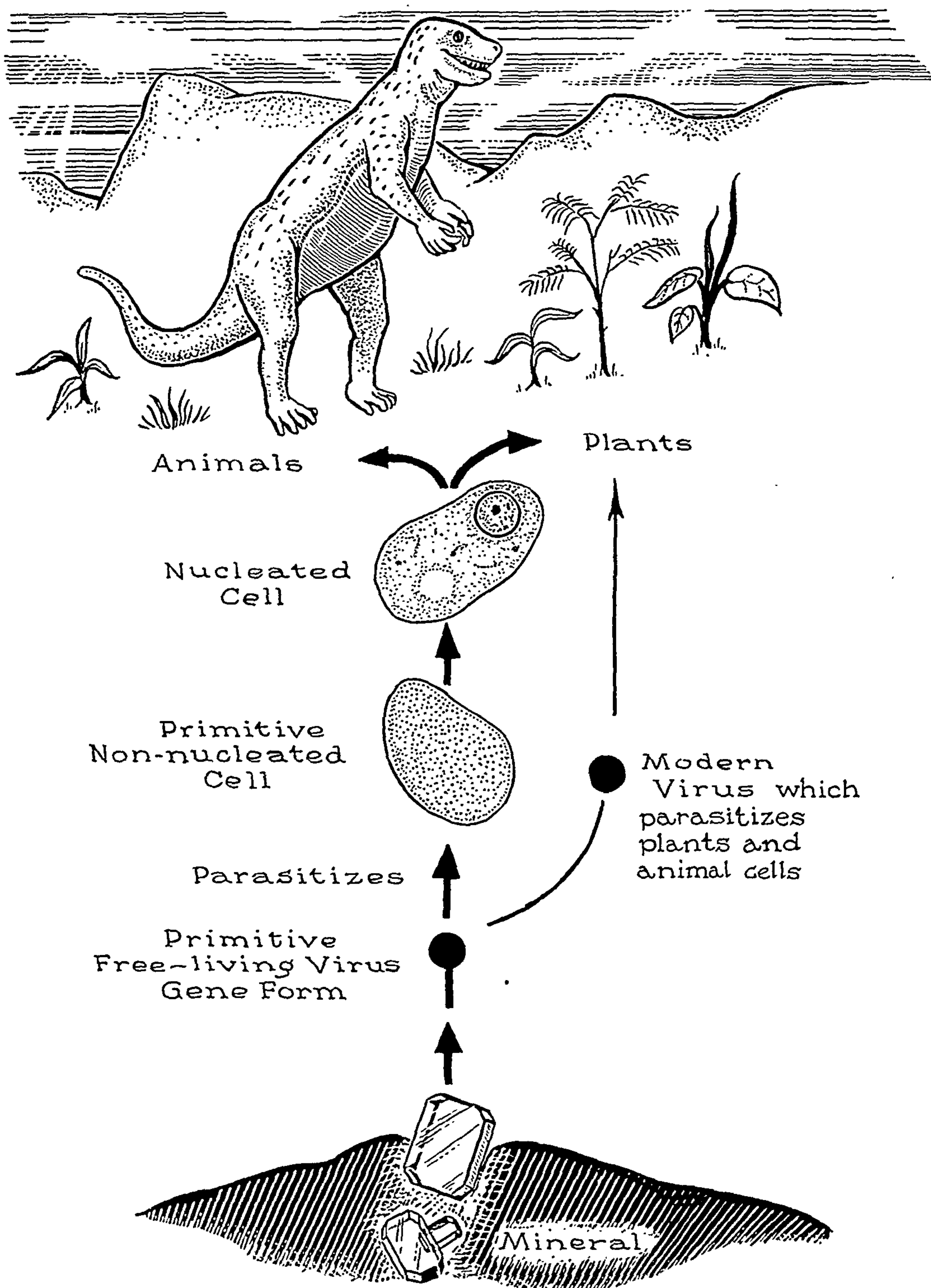
Transplantation of Tissues

VOLUME I



"Possible Evolutionary Chain from Minerals to Plants and Animals"

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Alternately, some of the primitive free-living virus gene forms give rise to the present-day viruses, which can be active and reproduce only within the bodies of susceptible plant and animal cells. Evolution of this primitive free-living virus gene form also gives rise to the more complex and larger viruses, rickettsiae, and bacteria. (Modified from Stanley.)

Transplantation of Tissues

CARTILAGE, BONE, FASCIA, TENDON, AND MUSCLE

Lyndon A. Peer, M.D.

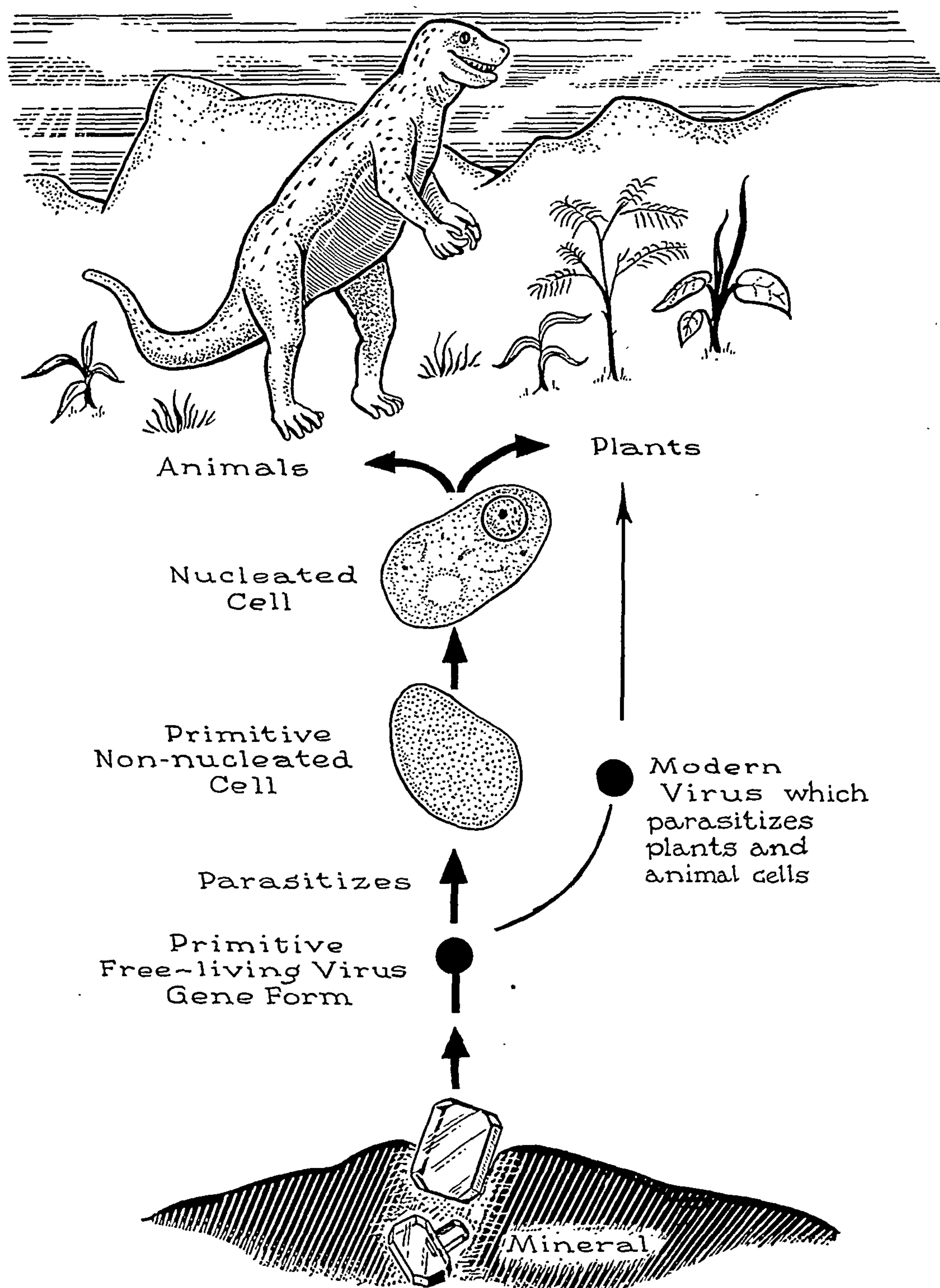
Clinical and Research Director, Rehabilitation Center for Plastic and Reconstructive Surgery, St. Barnabas Hospital, Newark, New Jersey

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Foreword

I can think of no one who is better qualified to present a book on Transplantation of Tissues than Dr. Peer. He is a happy combination of practical surgeon and scientific investigator, and thus is able to bring to his medical colleagues research information that will aid them in their daily tasks in the treatment and rehabilitation of patients.

An example of this ability to apply experimental findings for clinical use was demonstrated when Dr. Peer invented "diced cartilage grafts" after noting in human experiments that cartilage grafts became bound together in the form of a plaque after transplantation in abdominal fat. He was the first to use diced cartilage to repair skull depressions and other facial defects, and later devised methods which successfully utilized the multiple transplants in such diverse fields as ear reconstruction, ankylosis of the hip joint, spina bifida, recurrent abdominal and inguinal hernia, and large losses in the chest cage. Diced cartilage grafts and Dr. Peer's principle of introducing them into perforated vitallium molds to preform or shape the cartilage are accepted surgical procedures in many clinics here and abroad. He has examined and reported the histological findings in a larger number of autogenous and homogenous human cartilage grafts than any other investigator.

The book tells, as far as present knowledge goes, just what happens to various types of tissues, heterogenous, homogenous and autogenous, when transplanted into animals and humans. Dr. Peer has introduced each separate tissue, such as cartilage, bone, etc., with a preliminary chapter describing the structure of the tissue from a histological and surgical standpoint. He has also, I believe, for the first time succinctly divided all experimental work under that in animals and that in humans, sharply differentiating between the two, since what happens in the animal is not always exactly so in the human. After discussion of the basic experimental data relating to transplantation of each particular type of tissue, a chapter follows on the practical or clinical application of these data in the treatment of specific conditions.

The book correlates and evaluates a vast amount of material that has been heretofore scattered throughout the literature. This will make it a valuable tool both for those doing research work and for clinicians engaged in the varied fields of tissue, gland, and organ transplantation.

ROBERT H. IVY, M.D.

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Preface

It is now some thirty years since Harold Neuhof wrote his excellent book on "The Transplantation of Tissues," and until this time no attempt has been made to present a similar comprehensive treatise on the subject dealing with all varieties of tissues. During these decades research has brought tissue grafting into relationship with various other scientific fields, and a mass of experiemntal and clinical data has accumulated. It therefore appears timely to gather the material together, and evaluate its contributions to advancement in our knowledge of this dynamic and expanding science.

The form a book assumes is determined by the reader for whom it is intended. After considerable thought I decided to present the material in a manner designed to be readable and informative for the physician, surgeon, and medical student. This decision was probably wise, for my training and attitude are those of a clinical histologist, and all of my experimental work has been done with human transplants in human recipients.

Advances in medicine and surgery usually follow new concepts which are made possible by research information in different branches of science; when this information is correlated and applied for clinical use in man the new period arrives. It is evident that a new dynamic era of surgical replacement therapy utilizing the transplantation of tissues has now begun.

The already successful homotransplantation of embryonal endocrine glands in children and adults for the replacement of deficient glands, and the treatment of aging individuals who have lost their drive and interest in life are no longer fanciful. The possibility of permanent successful homotransplantation of skin in severely burned patients, the temporary success of whole organ transplants, the transplantation of embryonal teeth, and the clinical success of auto-, homo-, and even hetero-arterial grafts are becoming accepted advances in medical and surgical care.

It is now generally recognized that homogenous grafts of cornea, bone, and cartilage have a wide and expanding field of usefulness in clinical surgery. Diced cartilage segments either shaped in vitallium molds or introduced directly for such diverse conditions as hernia, new joint surfaces, and large losses in the thoracic cage have a wide application, which is not appreciated by the average clinician.

Thus it appears that we are on the threshold of a medical and surgical era which is destined to affect clinical practice profoundly. With a greater understanding of the behavior of cells, especially by employing tissue transplantation techniques, a new science that might be called "clinical histology" will probably develop. Because of the great advances in tissue transplantation today the science of "clinical histology" will logically become an integrated part of the medical college curriculum of the future.

In Volume I, the material is presented in a somewhat positive way for teaching purposes. The simplification, however, is based on microscopic evidence collected over a period of many years, and this evidence is correlated with clinical experience, and the gross appearance of the grafted area. Whenever my facts and theories differ from gen-

Contents

erally accepted opinions the latter are presented as accurately as possible.

Various additional aspects of cell structure and cell behavior will be described by a number of selected authorities in Volume II, which is to be published subsequently.

I wish to express my indebtedness to scientific friends for their interest and helpful counsel, and in particular to Miss Ruth Pullen, R.N., for her skilful drawings in clarifying the text, and to Miss Emma A. Buehler, B.A., M.A., for compiling the literature, editing, and her many constructive suggestions.

Dr. William G. Bernhard, director of laboratories at St. Barnabas Hospital, contributed valuable advice regarding the fixation of tissues and the microscopic interpretation of many sections. My former residents in plastic surgery all participated in portions of the experimental work (Dr. John Van Duyn, Dr. John Walker, Jr., Dr. Francis Marzoni, Dr. Max Pegram, Dr. Armand Genest, Dr. Robert Hagerty, Dr. F. S. Hoffmeistr, Dr. Clare Johnson, Dr. M. Shahgholi, Dr. Alvin Mancusi-Ungaro, and Dr. Blair Rogers).

The author welcomes this opportunity to express a deep sense of appreciation to Dr. George H. Lathrope of Morristown, and Dr. Royce Paddock of Newark, who have encouraged him in his clinical and experimental investigations over a period of many years.

L.A.P.

PART IV. FASCIA AND TENDON

PART V. MUSCLE

PART III. BONE

PART I

General Considerations

The Importance of Understanding Tissue Cells

The field of tissue transplantation is one of the most rapidly expanding frontiers of experimental and clinical medicine. Transplantation of tissues offers a wide range of new therapeutic advantages not only to the surgeon but to the internist, endocrinologist, cancer research worker, and many other scientists as well.

When properly chosen and applied, the newly-developed methods of tissue auto-, homo- and even heterotransplantation[1] furnish the physician and the research man with improvements upon preexisting forms of therapy and experimentation that open up innumerable new possibilities for hormone replacement, substitution of organs, growth of experimental cancer cells, etc.

It seems plausible that a knowledge of, and an interest in, the activities and biophysiologic environment of the several billions of cells that collectively make up the human being should help to enlarge the horizon and increase the understanding of the medical student and the physician, both of whom are concerned with the health of man.

Carefully controlled studies have already been made in which tissue-cultured embryonic adrenal and parathyroid glands have been transplanted to humans and to animals. These homotransplanted embryonic glands have survived permanently, taking over the functions of the diseased or absent glands. One patient with Addison's disease now survives without hormone injections as a result of having received such a transplanted embryonic adrenal gland.

Some patients have been relieved of hypoparathyroidism and tetany by the transplantation of embryonic parathyroid glands, but the cures and improvements have not always been consistent. *These successful results at any rate demonstrate that the science of homotransplantation is no longer in the realm of hypothetical speculation.*

Research in this field has developed to such a degree that surgeons no longer look with hesitancy upon the problem of how to transplant, for example, a kidney or a piece of skin but rather upon the problem of *how to induce these homotransplanted tissues to survive permanently*. The real crux of the matter lies in these words—"survive permanently."

One might say that no adult tissue exists which can be successfully and permanently transplanted from one human being to another, preserving its normal structure and

[1] Heterotransplantation has little if any clinical uses at this time. It is extremely valuable, however, in cancer and other forms of research. For instance, human cancer cells may be transplanted and studied in the rabbit and in other animals.

function intact in a living state. The successful experiments mentioned above were performed with the use of embryonic glands removed from stillborn babies.

There is a great need to solve the problem of homotransplanting adult tissues. One can see quite readily that many tissues would be ideally suited for use in permanent successful transplantations from one human to another, e.g., skin, kidneys, endocrine glands, bone, blood vessels, cartilage, cornea, hair, etc. Naturally, in most cases such tissues would be furnished by recently-deceased healthy young adults killed in accidents or by disease unrelated to the health of the tissues mentioned, or by infants who die as a result of unrelated disease. Because the supply of tissues from stillbirths is a limited one, the entire field of homotransplantation must, of necessity, approach the problem of the successful utilization of adult tissues. There is, of course, the possibility that embryonic tissues might be grown in tissue-culture media (a tissue-culture bank) and transplanted to children or adults whenever homotransplants were indicated.

There is also the possibility that small portions of tissue, such as skin, might be grown into large sheets in tissue-culture media and later applied as a covering autograft (in the same patient) to replace large skin losses.

Certainly further experimental work with autografts, which are generally successful, should be correlated with our expanding knowledge regarding the behavior of homotransplants. *The behavior of autogenous grafts is an important yardstick in evaluating the fate of similar homogenous transplants.* One might critically remark that both research workers and clinicians tend to undertake experimental and clinical application of homografts before they are informed regarding the known behavior of similar autotransplants.[2] This is not unlike plunging into pathology without a thorough knowledge of histology.

Research in tissue homotransplantation has already solved some of the difficulties which arise when the surgeon transplants a kidney or skin from one human to another. *The chief problem, however, of getting such a tissue to survive permanently is far from being solved.* As an example of how misleading information on this subject can be, the average surgeon and the average layman think that corneal transplants are generally successful. It must be remembered, however, that a good one-third of these transplants fail because of the so-called *"mal de greffe"* or graft sickness, a vague term which is used to describe the sudden onset of a series of events which cause destruction and clouding of the transplant, often within twenty-four hours. Is this sudden change for the worse due to an "immunity reaction" on the part of the patient directed against the foreign proteins in the transplant? Research is only now beginning to explore just such a possibility. This is important research, for one may make this categorical statement: *Whoever solves the method by which skin may be permanently transplanted from one human to another, preserving its normal architecture and its living cells* (perhaps by preventing this hypothetical "immune reaction" from taking place) *also solves the problems underlying the successful permanent homotransplantation of any tissue or any organ from one human to another.*

One may conclude from these examples given and from others not mentioned that future advancement in medicine will be closely associated with a knowledge of the structure and behavior of cells.

The physician who has a thorough understanding of the physiologic requirements of living cells is not apt to undertake surgical manipulations that are harmful or useless, nor will he prescribe drugs or other forms of therapy that adversely affect tissue cells.

Unfortunately, the average practicing

[2] There is much that is not known regarding the behavior of autografts.

physician and surgeon seems to have little knowledge of, and consequently little interest in, *the structure and normal activities of tissue cells.* At medical meetings and in hospital dining-rooms many doctors show an interest in discussions about abnormal cell activity when this is directly related to a patient's symptoms or is evident in the production of gross abnormal findings such as grapefruit-sized tumors or abscesses containing a liter of fluid. Interest fades, however, when the discussion turns to microscopic observations, and attention is directed to those small living entities that collectively form and maintain all human tissues. A discussion of physiologic and pathologic changes on the cellular level is too speculative for the average surgeon, who too often thinks of the structures that he manipulates in much the same manner as the craftsman who works with cloth or blocks of wood. The physician does give a fair amount of attention to the circulating system that nourishes and drains the tissues themselves, and usually he is aware of the importance of the cellular elements in whole blood, because these are vitally necessary for the control of shock or the success of an operation. He is not usually interested, however, in the cell groups that are actually supplied by the circulating system unless they are diseased.

It is difficult to determine just why a practicing physician should departmentalize his mind in such a manner that his early histological training in medical school is later applied to pathologic problems but is largely ignored when he deals with normal tissues. This, in part, may be due to the gap in teachings in medical schools between academic histology and pathology, on the one hand, and surgery and medicine, on the other.

Pathology deals with abnormal tissue cells. Because all pathologic conditions usually arise from normal cell groups, the structure and behavior of normal cells should be understood in order to better comprehend the abnormal cells. It is quite easy, however, to be so impressed with the *abnormal* that we lose our conception of *normal cell types*, and the physiologic requirements necessary for their survival and good health.

Thus, even after six years of postgraduate study in our stream-lined American Board system of training, senior surgical residents show an astonishing ignorance of the tissues which they manipulate, and of the requirements of living cells in these tissues.

It is an established fact that certain tissue cells when transplanted as free grafts tend to react in a specific manner. Some cells will always survive a favorable transplantation procedure, whereas some will only partly survive the transfer and others will never survive. Wide surgical undermining of tissues, relaxation incisions, or the shifting of tissues on attached pedicles will naturally affect the survival of cells, depending upon the extent to which their vascular and lymphatic circulations are interfered with, plus many other non-surgical factors. The cells in some tissues such as cartilage, skin, and fascia tend to survive free transplantation just as readily when severed from their original blood supply as when they are transplanted with an attached blood supply. The exact opposite is true, however, of the cells in fat and muscle grafts.

It is not uncommon to observe a skilled surgeon who performs an excellent operation from a technical standpoint but who transgresses one of the biological laws of tissue transplantation, with an operative failure as the result. A free fat graft improperly handled during an operation, for instance, will fail to "take" despite the skill of the transplantation procedure. Fat cells, as a point of explanation, are undeniably sensitive to even the slightest amount of trauma during a surgical operation. When poorly-handled fat grafts are transplanted to a new host site, host *connective tissue* will often replace the graft. In general, grafts will also fail if their

environment is radically altered. Thus, even skin grafts, which are composed of hardy, keratinized epithelial cells, tend to wither away when they are buried beneath the body surface. If these grafts are provided with even a small communication to the skin surface, they tend to survive, and as such they have been employed as a substitute lining—for example, in reconstruction of the urethra. Conversely, tissues such as fascia, tendon, and bone, which normally live buried under other body tissues, do not tend to survive when transplanted to the skin surface. With a better understanding of many poorly-known general facts about tissue transplantation the practicing physician and research worker would be in a better position to apply this knowledge to the perfection of improved methods for tissue grafting on both the clinical and laboratory level.

The entire field of free tissue transplantation has many important clinical applications. The doctor who practices in areas where there are no large medical institutions can still contribute valuable knowledge about the behavior of human tissue cells in transplanted grafts. The only equipment he needs is a compound microscope and his own individual powers of observation. When a favorable opportunity presents itself, with the consent of the patient or the patient's relatives, a tissue can be buried for varying lengths of time in the abdominal wall and later removed for microscopic examination. The conclusions drawn from such studies may be much more valuable than the numerous conflicting reports that now appear in the literature, and the fruits of such a study should have a stimulating effect on the investigator. Certainly in astronomy many significant contributions have been made by the amateur. Similarly the concepts and theories of a physician who does not consider himself an expert in tissue transplantation may be important; hypotheses are the soul of science and may be extremely valuable even if they eventually prove to be wrong.

The physician who develops this interest in normal and abnormal tissues and their transplantation will gain an understanding of cell behavior which can be applied clinically. In addition he may possibly help to contribute to the great fund of medical knowledge inherited from previous investigators, both living and dead.

CHAPTER 2

The Cell and Smaller Living Units (Viruses, Rickettsiae and Bacteria)

Man is a complex combination of highly specialized cells which, like all other living things, originated from a single cell form living in the sea. This single cell structure absorbed materials necessary for life from the liquid medium surrounding it, and excreted metabolic waste products back into this liquid medium.

CELLS

The Aquatic Environment of Cells

When some of these single cell forms became multicellular by means of evolutionary changes, the efficiency of their primitive methods of absorption and excretion was lost. New physiologic mechanisms or channels therefore evolved so that sea water could adequately nourish the smaller cell groups of the multicellular organism. These channels in larger multicellular organisms were altered to form a closed network known as the vascular system, but each cell figuratively remained aquatic and continued to be surrounded by intercellular fluid. Even to this day, in humans, chemically the intercellular fluid closely resembles the sea water that originally nourished the earliest primitive single cell structures. It is interesting to recall that the prehistoric oceans contained weaker salt concentration than at the present time; in this manner they more closely resembled normal physiologic saline.

In higher cellular forms, such as man, cells have retained their primitive method of metabolic absorption from, and excretion into, the intercellular fluid surrounding them, similar to the primitive single cell structures that long ago lived in the prehistoric seas. Thus, the survival and good health of every living tissue cell depend upon a circulation of fluid that resembles sea water. This fluid in closed vascular systems must diffuse through all anatomical barriers separating it from the individual cells.

The concept of the individual cell as the smallest unit of life and the development of a cell theory are not the results of modern scientific thinking. Early Greek philosophers, particularly Aristotle and Theophrastus, speculated that "all animals and vegetables are constituted by a few elements which are repeated in each one of them." While they were concerned with substances that could be seen with the naked eye, such as the roots, leaves, and trunks of trees, and the tissues and organs of animals, the basic concept of large structures composed of and arising from smaller elements was established.

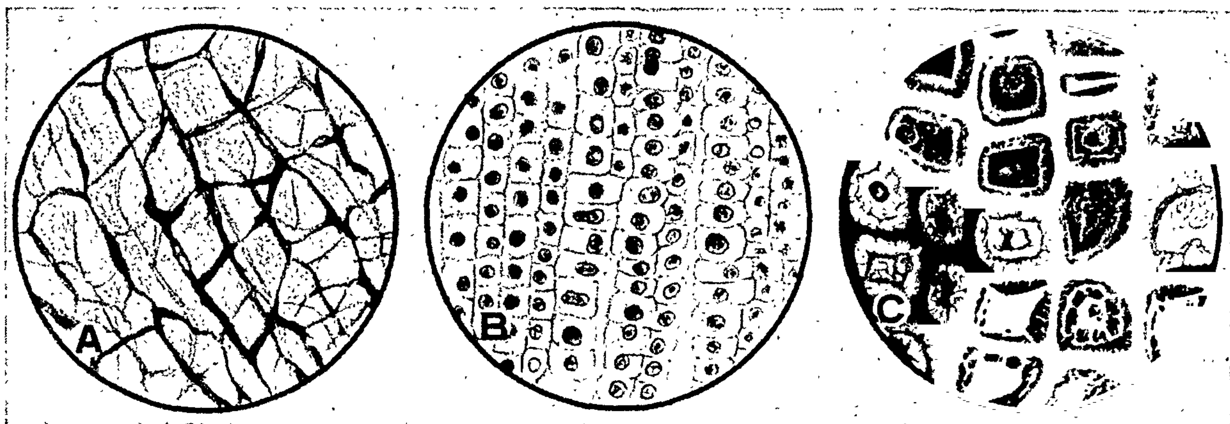


FIG. 1. At the left is a low-power photomicrograph of a slice cut free-hand through a piece of cork. This is probably what Hooke saw when he first described cells. In the middle is a medium-power photomicrograph of a longitudinal section cut through the growing root tip of the plant and it shows cellular residents in the compartments that Hooke described. At the right is a medium-power photomicrograph of a section of cartilage; it shows cellular residents in compartments of intercellular substance somewhat similar to those of plants. (Redrawn after Ham.)

The cell as a unit of structure was first described by Robert Hooke (1), an Englishman, in 1665. Examining the texture of a cork through magnifying lenses, Hooke observed that it contained many small compartments, arranged in a honeycomb-like manner. He noted the presence of limiting cell walls but gave little thought to "juice" or content of these cells.

The Cell Theory

The cell theory, which postulates that all plants and animals are composed of small units of life, was introduced by two German investigators, Schleiden and Schwann, in 1838 and 1839. They emphasized the significance of the jelly-like content of cells, which Hooke considered as a mere "nourishing juice" for the cell wall. Robert Brown, a botanist, in 1831, discovered the nucleus in cells, and Purkinje in 1839 gave the name *protoplasm* to the basic substance of animal embryos; von Mohl, in 1846, applied the term "protoplasm" to vegetable cells. Thus the gelatinous substance found in all cells became known as "protoplasm." From these original investigations the protoplasm theory arose, which states that the cell is an accumulation of a living substance—protoplasm—limited in space by a cell membrane and containing a nucleus. The cell is now accepted as the fundamental unit in the structure of both plants and animals, just as the atom is accepted as the funadmental unit in chemical structures. Obviously the atoms in the molecules in the cell are made up of protons, neutrons, and electrons. This makes cells similar to inorganic matter, with the important exception that a cell is *living* and inorganic matter is *dead*.

Recent studies utilizing polarization, diffraction, ultramicroscopic, and electron microscopic techniques have revealed many complex smaller structures in cells, bacteria, and rickettsiae. Microdissection and biochemical research have disclosed some of the possible functions and chemical composition of certain specialized particles found in the protoplasm. Thus the cell, which was formerly regarded as a simple structure, is now evaluated as an extremely complex center of diverse chemical activities.

Ultramicroscopy and electron microscopy now permit investigators to observe the "filterable viruses" that hitherto have been known only by their ability to pass through

fine filters or by the pathological alterations that they cause in human and plant tissue cells.

Haeckel, in 1868, suggested the existence of homogeneous proteins as the most primitive form of organic substance. Because of the inorganic-like chemical configuration of certain "mosaic" viruses, it is interesting to speculate that our present knowledge of these viruses seems to confirm Haeckel's theory, which suggests that the virus is a link between the inorganic and the organic world (see frontispiece).

VIRUSES

According to Stanley (2) there is no single criterion by means of which viruses can be differentiated from bacteria, but the virus has been segregated by means of certain general characteristics. Among the most important of these are its small size, the ability to reproduce or multiply within the living cells of a given host, the power to change or mutate during multiplication, and the property of reproducing or growing in artificial media containing susceptible host cells. Rivers (3) believes, despite statements to the contrary, that it is impossible to cultivate or induce a virus to multiply in the absence of living susceptible cells.

There is considerable controversy among biochemists and pathologists regarding the exact status of the virus, some believing that it is viable or alive, and others that it is non-viable or inanimate. It is difficult precisely to define "life," and the present controversy is not likely to be solved until a definition acceptable to all research workers is evolved.

Viruses vary in size from 300 to 10 millimicrons. Certain small viruses are smaller than the accepted protein molecules and, conversely, some large viruses such as the vaccinia virus are larger than some of the smaller living organisms.

Tobacco-Mosaic Virus

This virus, the first to be described, was also the first to be prepared in essentially a pure crystalline protein form by Stanley (4) in 1935. The tobacco-mosaic virus, shown to consist of approximately 6 per cent of nucleic acid and 94 per cent of protein, appeared as a rod-shaped anhydrous, crystalline structure.

It is both interesting and significant that tobacco-mosaic virus particles are completely devoid of water and of any enzymotic activity other than virus activity. A large number of other viruses that have been carefully studied appear to contain water—a characteristic of plant and animal cells, which require the presence of water for cellular activity and for survival. Human tissue cells definitely exist in an aqueous milieu; if the extracellular water surrounding them is removed, they lose their intracellular water content and die. According to Stanley (4), the complete lack of water and the crystal-like inner structure of individual tobacco-mosaic particles seem to rule out the presence of a metabolism of the type usually associated with living organisms. Yet when introduced into susceptible host cells these virus particles can direct or enter into the metabolic chain of events that take place in the cell.[1]

Animal Vaccinia Virus

Since Stanley's original work, other viruses have been crystallized and subjected to careful chemical analysis. Workers in Rivers' (3) laboratory have succeeded in isolating an animal vaccinia virus in crystalline form, demonstrating virus bodies

[1] Stanley's epochal work in demonstrating the crystalline structure of tobacco-mosaic virus has stimulated our current science fiction writers to bridge the gap between mineral and living structures. Thus, in some of their writings, an earthling visiting some distant planet is greeted by an intelligent anhydrous crystal who is part of a highly-developed civilization.

which are cuboidal in shape and contain several distinct proteins, lipids, carbohydrates, copper, biotin, riboflavin, and other substances. These elementary cuboidal virus bodies are surrounded by a membrane-like structure, demonstrable by its distention when treated with a dilute alkaline solution. This is followed by the appearance of breaks in the surface of this membrane through which, in some micrographs, a protoplasm-like substance can be seen in the act of streaming out, as reported by Green *et al.* (5). As interpreted by Rivers (3), the vaccinia virus possesses no respiratory, metabolic or reproductive activities in the absence of living, susceptible host cells. The accumulated information about the vaccinia virus (3) furnishes strong evidence that at least one animal virus is a complete structure quite different in composition from the plant tobacco-mosaic virus, which consists only of nucleoprotein.

Small versus Large Viruses

In general, the smaller viruses seem to have more primitive or simpler structures than the larger viruses, whose complexity of composition, structure, and function increases with their size. Because of this apparent direct relationship between the size of a virus and its complexity, *it has been suggested by Stanley that the viruses provide a link between inorganic molecules and organisms, creating an evolutionary pathway leading from simple elements such as the electron, to massive, highly complex structures such as man.*

Virologists disagree as to whether or not the smaller viruses, particularly the crystalline ones, are living structures. Some believe that they are non-living and represent end-products manufactured by their host cells (6) through autocatalytic processes. Other virologists endow them with a sort of "half-life," between the living and the non-living state.

Whatever virologic concept proves to be correct, the scientist of today cannot ignore the possible role that viruses might play in evolution merely because he cannot put his finger on the exact evidence that will permit him accurately to define the terms "life," "half-life," and "inanimate."

In science as in religion, when all is known, all will be understood. *The metaphysical of today often becomes the science of tomorrow.*

GENES

Direct chemical analysis of whole chromosomes demonstrates that they are largely composed of nucleoprotein (7), which suggests that the genes also probably contain a large amount of nucleoprotein. Thus, a similarity in structure could be drawn between the gene and the more primitive smaller viruses, which are also composed largely of nucleoprotein.

Another similarity between genes and viruses is their power of self-duplication, which is dependent upon the presence of certain substances found in living cells. Both genes and viruses multiply only within specific cells where certain necessary substances are available and the environment is satisfactory.

Genes and viruses are also within the same general size range (8), and both can undergo mutation, giving rise to new forms which have altered biological activities. These new forms, moreover, retain their power of self-duplication (8).

Evolution and Primitive Gene-Virus Forms

Troland (9), Oparin (10), and Muller (11), among others, recognizing the similarity between genes and viruses, suggest that the earliest living structures in evolution with the ability to reproduce themselves were probably somewhat similar to present-day viruses, with the exception that they were *free living.* It is possible that this virus was destined to give rise to higher forms of life

such as cells, which are now parasitized, at a later stage of evolution, by the progeny of these early virus forms.

Evolution from this primitive free-living virus gave rise to the more complex and larger viruses, rickettsiae, and bacteria as well as to the higher organisms. Present-day viruses may represent forms which have been derived because of specialization in connection with parasitism (12); they may not closely resemble the primitive free-living virus. The true ancestral types might have been able to multiply outside living cells in an environment which, due to the presence of innumerable types of organisms today, is no longer apt to exist (10).

The genes may have also evolved from simple free-living forms, developing into the more highly specialized forms which now exist only within cell nuclei (13).

We have discussed the similarity between viruses and genes in their biochemical structure, size, property of self-duplication, and ability to mutate. One important difference, however, is the fact that the modern gene regardless of the habits of its hypothetical free-living primitive ancestor, can exist only within a living cell, whereas the virus can exist outside of it.

Genes and Cells

The methods by which the early hypothetical free-living gene became incorporated in the nucleus of the cell is a matter of speculation. It is possible that a primitive free-living virus-type gene parasitized a primitive non-nucleated cell and, through cooperative co-existence with the cell, established a symbiosis. Subsequent evolution of this primitive gene within the parasitized cell may have resulted in the more complex nucleated cell found in plants and animals (see frontispiece). *That in certain instances there is a cooperative effort rather than a destructive effort between the present-day parasitic virus and the parasitized cell is an established fact.*

As virologic, genetic, and immunologic research findings accumulate, *it seems apparent that biological evolution seems to favor the development of single forms into more and more complex structures.* This biological law probably applies equally well to the foregoing hypothesis: that early precellular forms developed successively into the primitive cell, and the cell into the highly complex entity of cells that make up the human being.

RICKETTSIAE AND BACTERIA

A number of human infections are caused by microorganisms called "*rickettsiae.*" These are intermediate in size and characteristics between bacteria and viruses. Studies with the electron microscope reveal the structure of rickettsiae to consist of an apparent limiting membrane surrounding a protoplasm-like substance which contains a number of dense granules. A distinct and recognizable nucleus has not been observed. The rickettsiae are visible in microscopic preparations as coccobacillary forms and, like viruses, they multiply only within susceptible cells.

Bacteria were formerly thought to be the simplest and lowest of all living forms, beyond which life did not exist. Examination in previous years with the ordinary compound microscope failed to reveal a nucleus or any other structure within the substance of bacteria. They were therefore considered as being mere bags of enzymes with great biochemical activity rather than true cells. But examination with the electron microscope has demonstrated the presence of bodies within bacteria that apparently are equivalent to the vesicular nucleus in typical cells (14). Hence a bacterium is now called a bacterial cell. Some botanists regard the bacteria as plants, which, like fungi, do not contain chlorophyl; others believe that they are intermediate forms between plant and

animal. The lower the evolutionary phase of development—if one considers man as the highest form of development achieved to date—the more difficult it becomes to decide whether a specific organism belongs to one biological kingdom or another. Many living things combine both plant and animal characteristics in a manner most embarrassing to those who wish to pigeonhole or categorize all living forms either as plants or animals. Bacteria have some of these irritating character combinations.

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CHAPTER 3

The Human Tissue Cell

Although the theory that all plant and animal organisms are composed of cells is associated with the names of Schleiden (1838) and Schwann (1839), numerous investigators had previously proposed this same theory in a more or less complete form (1). The findings of Schleiden concerning the constitution of living matter in plants were confirmed in and extended to animals by Schwann who, for the first time, used the term "cell theory" for the concept that animals as well as plants are aggregates of cells arranged in accordance with definite laws. The cell theory was then rather quickly applied to explain the structure of unicellular organisms, spermatozoa, and that of the ovum, from which—by division of cells—the organism is developed.

The main difference between plant and animal cells is that the former contain chlorophyl, which is so vitally sustaining that the food it synthesizes supports all organic life on the earth. Certain plants, such as the fungi, do not contain chlorophyl; but those which do not have it must steal its products from other plants in order to exist.

The cell principle includes two concepts: 1) that the bodies of all plants and animals are composed of cells and the products of cells, and 2) that new cells are derived only by the division of preexisting cells. According to Coulter (2), all modern plants have probably been derived from one or a few very primitive ancestral types by a process of gradual modification through descent. How these early ancestors were first brought into existence is another question—a question for which no one has found a satisfactory answer. Biologists, speculating on the problem, have come to favor certain hypotheses. The most plausible one seems to be that the first living organisms upon earth were derived from non-living materials already present, but just how this occurred remains a mystery, for man has not been able to produce life from non-living materials or to discover anything of the sort occurring spontaneously in nature today. Coulter suggests that perhaps the different conditions of the remote past were responsible, and man, in his few and too brief experiments, has not as yet properly duplicated these conditions. On the other hand, it may be that today life sometimes starts to originate as it did in the past but the living element is regularly consumed, before it can be observed, by the ubiquitously established organisms of modern times (see frontispiece).

CELL DIVISION

The cell perpetuates itself by cell division, which is a universal activity in our world. Through the process of cell division the chromosome splits lengthwise, half moving

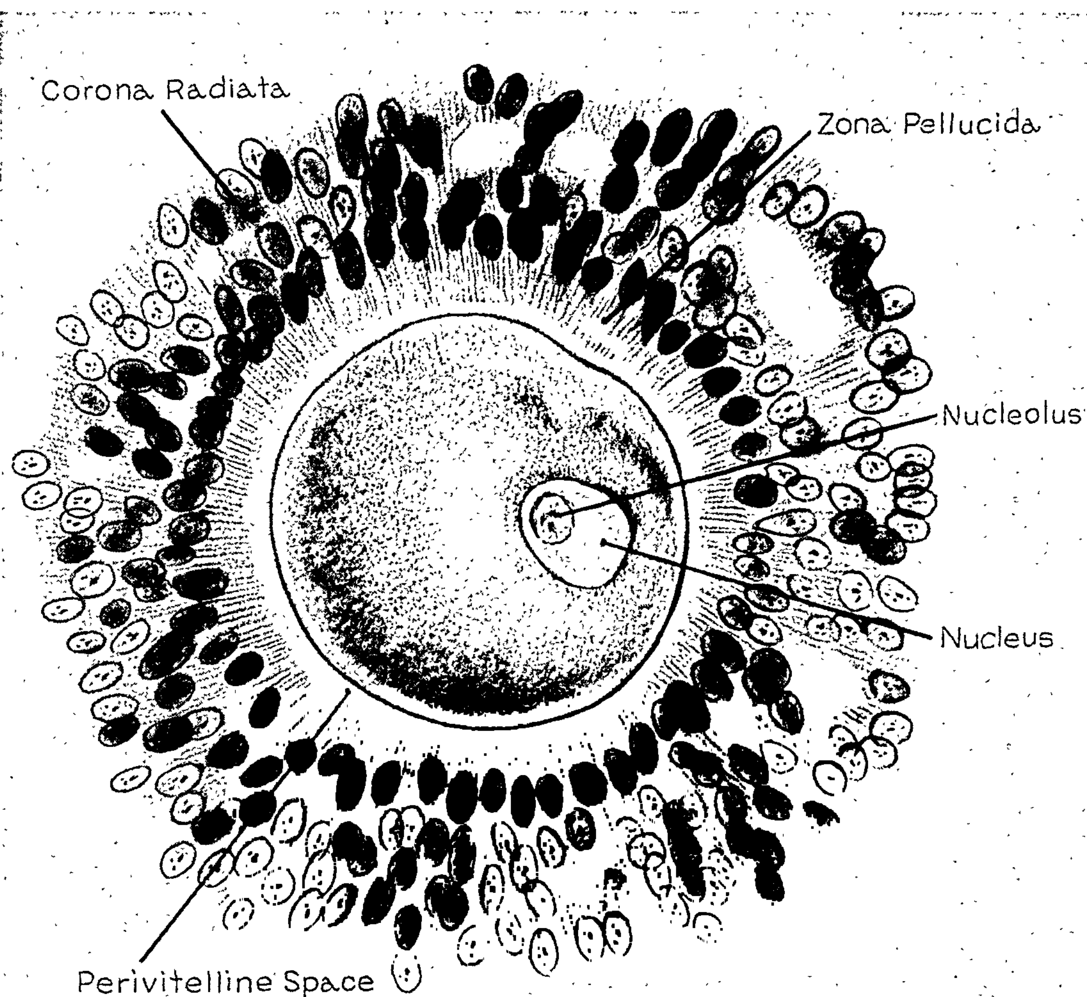


FIG. 2. Human female sex cell. (Redrawn after Bumm.)

toward one pole and half toward the other. A new cell wall cuts the two groups across the middle and the two halves separate, forming two cells. In this way every cell is part of a series, or a unit in the sequence of reproduction, which is a sort of immortality. The ovule of the sequoia tree and that of the human are surprisingly similar when viewed under the microscope, but each is endowed with different growth potentials, so that through cell division the former gives rise to a giant tree, whereas the latter produces a man.

Growth of the human individual, as is well known, is effected by consecutive cell division called mitosis. At first this growth is rapid, the germ cell dividing into two daughter cells, which in turn will produce a generation of four, then eight, sixteen, thirty-two and so on. By simple arithmetic we can estimate that fifty to sixty successive divisions can produce the number of cells in a grown human, which is about a hundred or thousand billion. The human tissue cell is therefore only about the fiftieth or sixtieth descendant of the egg from which the human evolves.

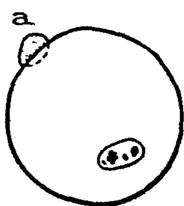
PLASMA OR CELL MEMBRANE

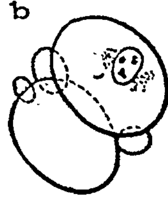
The cell membrane, first described by Hooke and later appraised by Schleiden and Schwann as a mere sack containing protoplasm, is a complicated and important structure which controls the entrance and

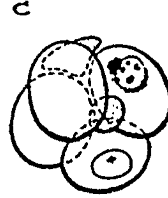
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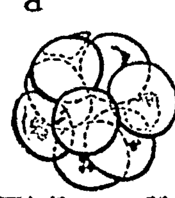
OUTLINE DRAWINGS OF THE CLEAVAGE STAGES IN THE MONKEY (*MACACUS RHESUS*) x ca. 150

a, Corner (1923) b, c, d, Lewis and Hartman (1933)







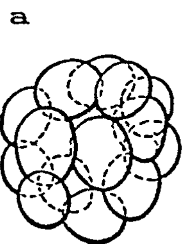


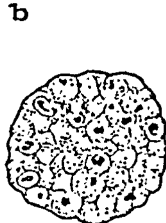
+time after ovulation

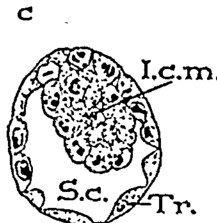
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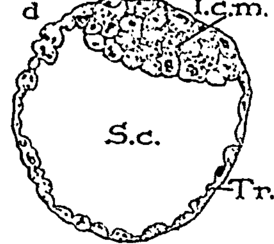
MORULA AND BLASTULA STAGES OF THE RABBIT OVUM x ca. 200

Gregory (1930)









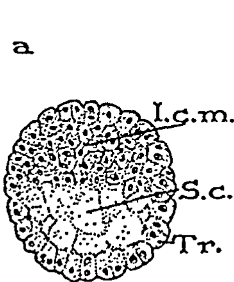
a. Living morula of ca. 32 cells
b. c. d. Sectioned blastulas

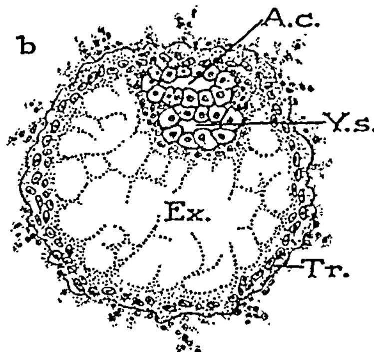
I.c.m. - Inner cell mass
S.c. - Segmentation cavity
Tr. = Trophoblast

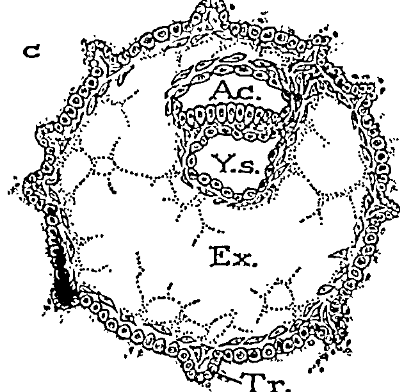
C

DIAGRAMS OF THE POSSIBLE COURSE OF EARLY DEVELOPMENT IN MAN

Based on the schemata of Patten (1933) and Scammon (1922)







A.c. = Amniotic cavity
Ex. = Extra-embryonic coelom
I.c.m. - Inner cell mass

S.c. = Segmentation cavity
Tr. = Trophoblast
Y.s. - Yolk sac

FIG. 3. Segmentaion of the ovum and early development of the embryo. *A, Outline drawings of cleavage stages of the ovum of the monkey (Macacus rhesus).* (*a*, From Corner, 1923; *b*, *c*, and *d*, From Lewis and Hartman, 1933.) *B, Morula and blastula stages of the rabbit ovum.* (From Gregory, 1930.) *C, Diagrams of the possible course of early development in man.* (Based on the schemata of Pattern, 1933, and Scammon, 1922.) From Morris' Human Anatomy, 11th ed., Edited by J. Parsons Schaeffer. New York, Toronto: Blakiston Company, 1953.

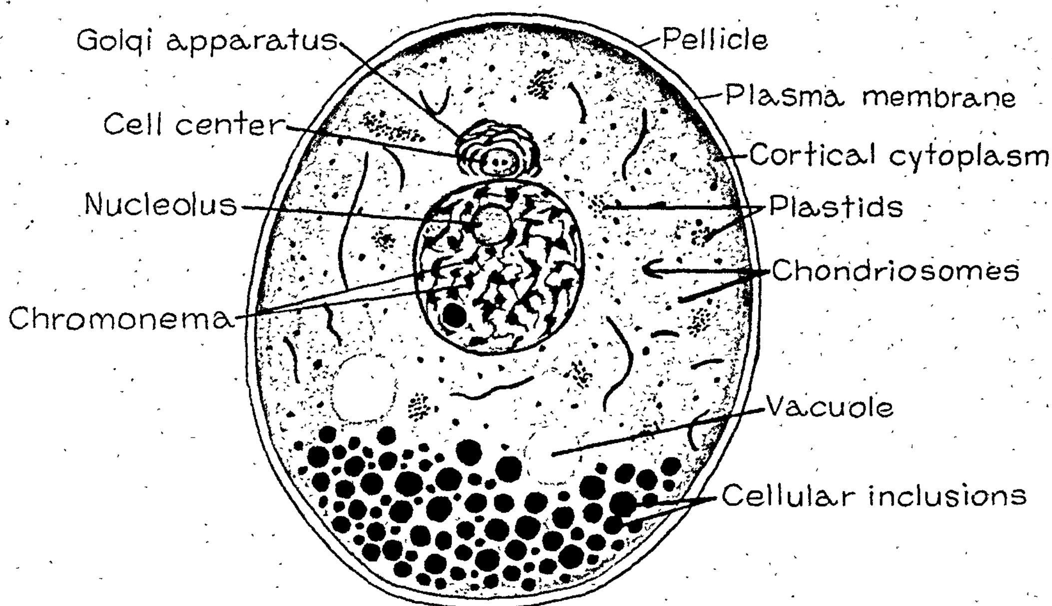


FIG. 4. Idealized drawing of human tissue cell

exit of all substances necessary for cell metabolism. The cell membrane is so thin that it is beyond the limits of microscopic visibility with ordinary light; it is visualized as the surface of separation between the cell content and the tissue fluid outside the cell. Special studies indicate that the cell membrane consists of a continuous layer of lipoid molecules arranged radially to give permeability, with absorbed layers of protein molecules arranged tangentially to provide tensile strength.

The ionic content of cells, which may differ from that of the surrounding fluid medium, is maintained throughout the life of the cell by continuous control, by the cell membrane, of the entrance and exit of molecules and ions. The cell membrane is permeable to both water and some solutes, but the passage of various other solutes through the membrane does not occur with the same facility. In general, osmotic pressure is preserved by a mechanism which regulates the concentration of dissolved substances within the cells. In humans the regulation of osmotic pressure as a whole is effected mainly by the kidneys and sweat glands.

FLUID ENVIRONMENT OF CELLS

The tonicity of the fluid surrounding the cell is of vital importance for normal cell function and, indeed, for cell survival.

This fluid environment in which cells live has been emphasized by Cowdry (3), who says: "Any concept of cell life which ignores the fluid environments of cells and the roles of the three membranes, endothelial, cell and nuclear, is narrow indeed." Certainly the basic integration of our billions of cells is nicely regulated by water-borne traffic. It is important to remember, however, that the fluid surrounding the cells must have the same osmotic pressure as the cells; this is provided by a solution of 0.951 per cent sodium chloride. Solutions of greater or less concentration should never be brought in contact with living tissue cells during a surgical operation.[1]

[1] One may demonstrate tissue injury through disagreeable nerve response by dropping distilled water into the nose and comparing the sensation with that produced by a normal saline solution. It is not too fanciful to suggest that fresh wounds be irrigated with normal saline rather than tap water or antiseptic solution, which injure exposed tissue cells, making them susceptible to the action of bacteria.

The human tissue cell is essentially an aquatic organism, in this respect resembling the single-celled structures living in the ocean and in fresh water ponds. Both absorb through their cell membranes from the surrounding liquid medium the materials which they require, and excrete similarly through the membranes to the medium waste products which they desire to be rid of. Most of the several billions of living cells constituting the human individual are surrounded by interstitial fluid, and living cells on the body surface, in contact with air, are protected by moist films as in the eye, nose and mouth. Surfaces not covered by moist films are protected by layers of dead cells, as in the epidermis, which prevents the living cells from drying out. We humans, who consider ourselves land creatures, surrounded by air which we breathe, are composed of cells which exist and can exist only in a liquid environment if they are to remain alive.

The continuous activity of absorption and excretion usually takes place through the simple-appearing cell membranes. An exception to this statement is found in the excretory method of gland cells in the pancreas and thyroid, and in the excretion of collagenous material by the fibroblast, as described by Stearns (4). In such processes excretion is accomplished by a pinching off of the plasma membrane containing the secretion, somewhat similar to a minor cell division. The point where the surrounded cytoplasm pinches off remains covered by plasma membrane.

CELL CONTENT

The plasma or cell membrane both surrounds and contains the jelly-like protoplasm, first described by Schleiden and Schwann. This protoplasm is composed of two structures, the cytoplasm and the nucleus, which are the sites of internal specialization occurring in cells. Since the nucleus was discovered by Brown (5), in 1835, to be a constant part of the cell, cytologists have been interested in its activity and behavior. Modern studies have demonstrated the constant presence of the nucleus, or its equivalent, in every living cell, and its important role in cell activities. The nucleus appears to be concerned mainly with growth, the reproductive cycle, and the imparting of character. The cytoplasm controls simpler cell activities such as secretion and excretion, phagocytosis, absorption, and contractility. The dominant part played by the genes during cell division is well known, but an account of the numerous structures in the cytoplasm and nucleus will be cheerfully turned over to others. Certain basic conceptions, however, are essential; among these is the fact that the nuclear membrane, through which substances from and back into the cytoplasm must pass, is important for the survival, reproduction, and functioning of the human tissue cell.

Very little is known regarding the molecular composition of nuclear membranes but they must be rather tough, since intact nuclei can be centrifuged and collected after rupture of the cell membrane and removal of the cytoplasm. Cytoplasm has the ability of healing itself after moderately-sized rupture in the presence of the calcium^{++} ion. This property is not possessed by the nucleus, so that when the nuclear membrane is broken, its content flows out and the nucleus collapses without showing any tendency to repair (6).

The interrelationship between the nucleus and the cytoplasm is necessary for the activity and life of a human tissue cell. Experiments with fragments of cells without a nucleus have demonstrated that the denucleated cytoplasm survives only a short period of time and is not able to grow or reproduce. Nuclei themselves cannot live as isolated entities since they require a certain quantity of cytoplasm in order to be maintained (7).

It should be emphasized, however, that a

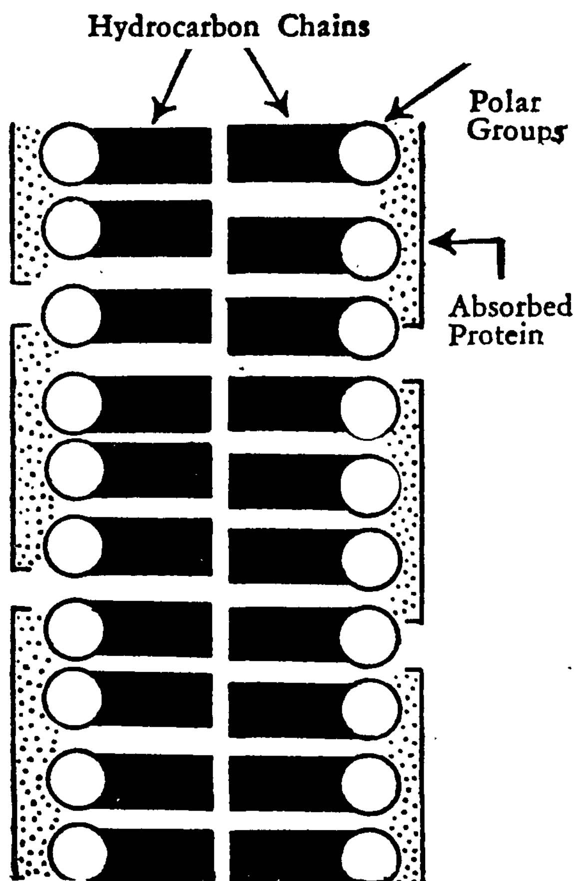


FIG. 5. Diagram of the molecular structure of the plasma membrane. (After Danielli.) According to this theory the membrane is constituted by a lipoid layer of small molecules, with a protein layer adhering at the lipid-aqueous interface. As shown in the diagram, the lipid layer is conceived as being bi-molecular, with the polar groups situated at the lipid-aqueous interface, while the non-polar groups are adjacent to each other. From General Cytology, 2nd ed., p. 161. E. D. P. De Robertis, W. S. Nowinski and Francisco A. Saez. Philadelphia: W. B. Saunders Company, 1950.

great amount of work is being carried out in the fields of microdissection and microtransplantation. In the future it may be possible to transplant nuclei from human tissue cells successfully into suitable media or favorable host cell sites which permit the nuclei to survive. The autogenous intracellular transplantation of nuclei, centrioles and the like into cells of a similar type and into the cytoplasm of different cell types has many interesting possibilities.

FORM AND SIZE OF CELLS

The different human tissue cells vary in size and shape, depending on their type and also somewhat on their location and phase of activity. Common characteristics of all living cells, however, are that they have a plasma or cell membrane, are suspended in a fluid medium, will die if the fluid is withdrawn, and contain a nucleus which is separated from the surrounding cytoplasm by an important structure, the nuclear membrane.

Authorities do not accept reports that desiccated cells from the tissues of Egyptian mummies can be revived and grown in tissue culture. The majority of authorities also doubt that the cells in dried frozen human tissue can again become viable although some very recent investigations indicate that it may be possible.

Among the many errors that die hard is the sprouting of dried wheat found clutched in the hands of Egyptian mummies. Sir J. Arthur Thompson (8) writes: "Man dearly loves a touch of the magical, and he is unwilling to give up the picturesque belief that wheat from inside a mummy-case may sprout after thousands of years of dormancy. There is the story of a man who bought some 'mummy-wheat' in Egypt and sowed it in Australia, where it germinated and grew with great vigor. There are many such 'records' but in every carefully conducted scientific experiment the true mummy-wheat has refused to sprout at all. What happens in the ordinary popular experiments is the sprouting of faked mummy-wheat, that is to say, the substitution of modern seeds for the ancient ones. The alleged mummy-wheat sometimes grows into a variety that was not known in the times of the Pharaohs but evolved in the early twentieth century."

Some weed seeds, such as those of the wild morning glory, however, have the ability to sprout and grow after lying buried for thirty years. According to Hottes (9), Robert Brown in 1843 tested some seeds of the lotus that were 150 years old; fourteen seeds germinated and two failed. Dr. Ohga, a Japanese botanist, succeeded in germinating some seeds dug from a Manchurian peat bog, where discovery of the plant had not been recorded by other botanists. Dr. Ohga considered these seeds 400 years old, yet they germinated 100 per cent.

To prolong the duration of a seed it is quite necessary that the seed be kept perfectly dry. In the case of lotus the drying of the embryo (seed content) is partially a chemical one, according to A. W. Exell of the British Museum, and water is used up from the inside in the thickening wall which surrounds the embryo. On the other hand, this seed coat also retains the moisture so that seeds do not become too dried to retain their life forces.

Human tissue cells have a single nucleus with the exception of skeletal or voluntary muscle cells and the megakaryocytes in the bone marrow, both of which are multinucleated.

Cells living freely in their fluid medium, as in the blood or lymph, and not closely packed together, often have rounded contours. An exception is the red blood cells, which are elastic biconcave discs containing hemoglobin. These cells, however, have lost their nuclei and are non-living bags of oxygen-carrying hemoglobin. Cells that are closely packed together, such as epithelial cells, are roughly hexagonal in shape.

Although different types of cells vary as to size and shape, there appears to be a general uniformity among those of similar types. Some, like fat cells, are capable of great enlargement through storage of additional fat, whereas in starvation the fat content is diminished or lost and the cell becomes much smaller.

Fibroblasts also may attain considerable size on engorgement, while in dense scar and keloids they appear as very thin elongated structures, almost lost among the thick rope-like masses of homogeneous substances and fibers which they have produced or with which they are associated.

Striated or voluntary muscle cells, which are multinucleated, are among the largest cells in the body, being several centimeters in length and often thick enough to be seen with the naked eye (10). Recent experimental work (11) has demonstrated that the muscle cell or fiber may extend from the origin to the insertion of a skeletal muscle. Bloom (12) in 1952 summarized the known facts regarding the length of voluntary muscle cells as indicating that the muscle cell or fiber is much longer than was formerly believed. The only other cells of equal girth and visibility are the eggs or ova (Cowdry). Small lymphocytes are among the smallest cells in the body, and nerve cells are the longest—but so thin that they are quite invisible.

A question which naturally arises is: why must cells be so small? From a physiological standpoint this may be answered as follows. The cell absorbs substances necessary for its metabolism from the fluid medium in which it lives, and excretes waste products of metabolism back into its aqueous environment. Both absorption and excretion occur at the surface of the cell membrane, and it is essential for the surface of the cell to have sufficient contact in order to provide efficient exchange for the mass of the cell body. An efficient balance of metabolism is obtained in small rather than large bodies.

Circulatory System. Most thick masses of cells and intercellular substances comprising tissue have developed a vascular system which supplies all living cells with their necessary aqueous medium through small tubes, the capillaries. Free massive transplants such as large fat grafts are probably kept moist but not nourished adequately in their central parts by the surrounding tissue fluid. Survival of such bulky grafts is a race for reestablishing circulation through the vascular system of the graft before the centrally located cells die from concentration of waste products. The new blood supply is provided in about three days after transplantation, by means of direct anastomosis between host and graft blood vessels. Later a penetrating ingrowth of capillaries from the host tissue also occurs, and this in all probability eventually anastomoses with the established circulatory system in the graft, but proof that this actually takes place is lacking.

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CHAPTER 4

Differentiation, Aging and Death of Tissue Cells

The higher organisms develop from a single cell—the fertilized ovum—which in the human through fifty or sixty successive divisions gives rise to all structures in the body. At first the process is a simple increase in the number of cells but in man, after the blastula has been formed, the cell divisions become qualitative and three germ layers are established. These three germ layers through further differentiation give rise to all tissues and organs.

CHANGES IN DIFFERENTIATION

In this process of differentiation the nucleus, which plays such an important part in heredity, changes very little. The cytoplasm, however, may differentiate greatly, giving rise to the fibrillae in muscle cells, which have the property of contraction; the axons of nerves, which represent a sort of cytoplasmic extension or tail; the ingestion of fat, resulting in the swollen fat cell and so forth. The cytoplasm also plays a dominant role in producing the inanimate intercellular substance, giving rise to or associated with the production of such different dead substances as are present in bone, tendon, and cartilage, which have large amounts of intercellular substance and relatively few parenchymal cells. Thus, most directly through activity of the cytoplasm, the cells are sorted out into the three germ layers—the ectoderm, mesoderm and endoderm—which through further differentiation form the various tissues and organs in the human body.

One region of the amphibian embryo was shown by Spermann and Mangold (1) to be determined at a very early stage—possibly at the time of fertilization—and it is, therefore, very much less plastic than the rest. This is the dorsal region or lip of the blastopore, which in the later stages of gastrulation, gives origin to the notochord and mesoderm (see fig. 6., chapter 4.). The dorsal lip region develops "selfwise" and in no direction other than to its normal presumptive fate. Further, if all or part of it is grafted into the undetermined tissue of another blastula or early gastrula, the neighboring host cells, irrespective of their presumptive fates, are brought to form or to attempt to form the tissues of a secondary embryo. On the other hand, in the absence of the dorsal lip region from an early embryo, normal development does not occur. Since these blastopore cells apparently contain within themselves the ability to determine the behavior of regions with which they are in contact, Spermann called the dorsal lip

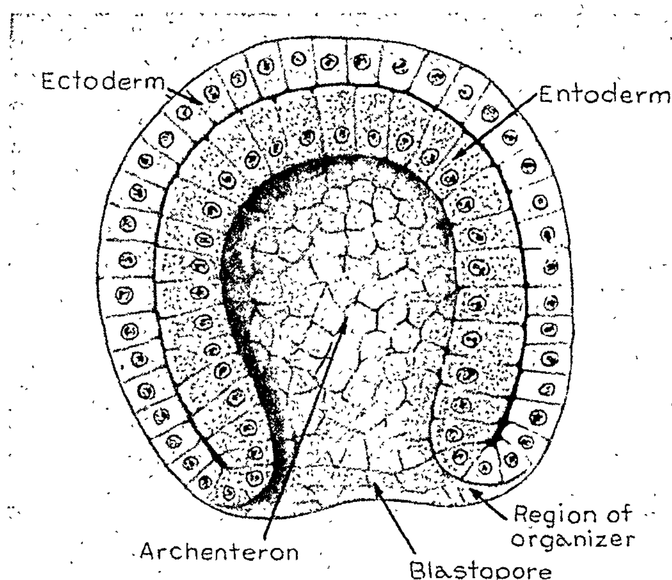


FIG. 6. Blastula stage of human ovum indicating region of organizer which causes specialization among cells. (Redrawn after Arey.)

region the *primary organizer*, it being the first of such mechanisms to function in development.

While the concept of primary, secondary and tertiary organizers has played a useful role in the study of development, recent opinion indicates that it is an over-simplification of the complicated phenomenon (2).

The activity of the primary organizer is presumably due to the presence of a chemical substance in the cells of the dorsal lip region. According to Hamilton *et al.*, this substance can be regarded as a morphogenetic hormone which passes by diffusion from the blastopore cells, or their developmental derivatives, to other cells where it modifies the rate of development and the direction of differentiation.

The life expectancy or normal life span of the different human tissue cells varies greatly. In nerve cells, for instance, after development in early infancy no new nerve cells are produced. This is true also of heart muscle. The red blood cells, however, have a chemical life span of about two months and are constantly being replaced by nucleated cells in the bone marrow. The cells in the basal or germinal layer of the epidermis as well as those in the more superficial stratum lucidum are continuously undergoing cell division throughout an individual's life; in this manner they produce the horny surface layer of dead cells, which retain the aqueous medium surrounding the deeper living cells. The life span of cartilage cells after they stop dividing is not known. This also applies to fat cells, tendon and fascia cells, bone cells, and others.

One should bear in mind that apparent growth of such structures as cartilage, tendon, fascia, and other tissues may take place by an increase in the intercellular substance without increase in the number of cells through cell division. The intercellular substance of cartilage, presumably elaborated by the cells, may increase the bulk of the cartilage substance and separate the cells one from another but not affect the number of cells. Cartilage in infants and young children appears to have more cells per area than does adult cartilage.

The growth of cells may take place either by enlargement of individual cells or by the multiplication of cells by cell division. Cell division is necessary for continued cell growth, for otherwise the cell would soon reach a size where its surface would be inadequate (for nutritive, respiratory and ex-

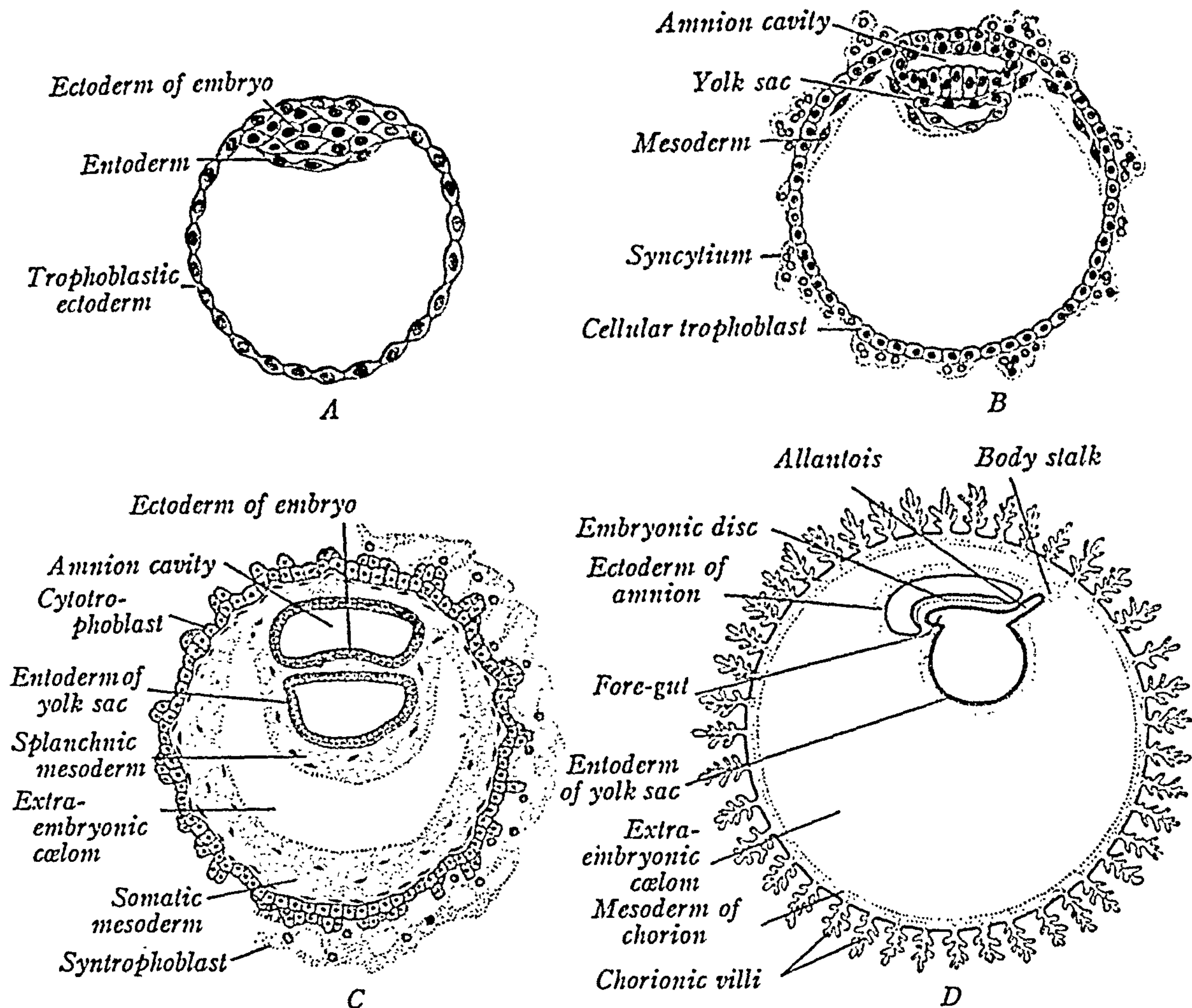


FIG. 7. Diagrams of human embryos of ten to twenty days. From Developmental Anatomy, Leslie Brainerd Arey, Ph.D. Philadelphia, London: W. B. Saunders Co., 1941.

cretory purposes) to its mass. In general, however, cell division is most active in the early embryonic periods, during which the cells remain small. Later, cell division diminishes or ceases in some parts of the body and growth is due chiefly to the enlargement of cells already present. The growth of the structural units of organs also follows this general rule, the production of new units being confined mainly to fetal and early postnatal life (3).

THE AGING PROCESS

After differentiation occurs, almost all cells pass on to a phase of aging or senescence, which ends in death. Throughout the individual's life many cells are aging and dying, as illustrated by the constant death and replacement of red blood cells and by the disintegration of the external horny layers of the epidermis. The death of the individual, on the other hand, is not associated with immediate death of all tissue cells. Leukocytes continue their amoeboid activity and the cells of the trachea continue their ciliary movement long after the heart has stopped beating. Death of the individual, however, eventually results in the death of all cells unless they are transplanted into tissue cultures or into the tissues (cornea, lens, and cartilage) of living humans (4).

A section of human rib cartilage was transplanted beneath the chest skin of another living patient. This homograft was removed three and a half years later, and on section showed only a moderate amount of absorption at the periphery.

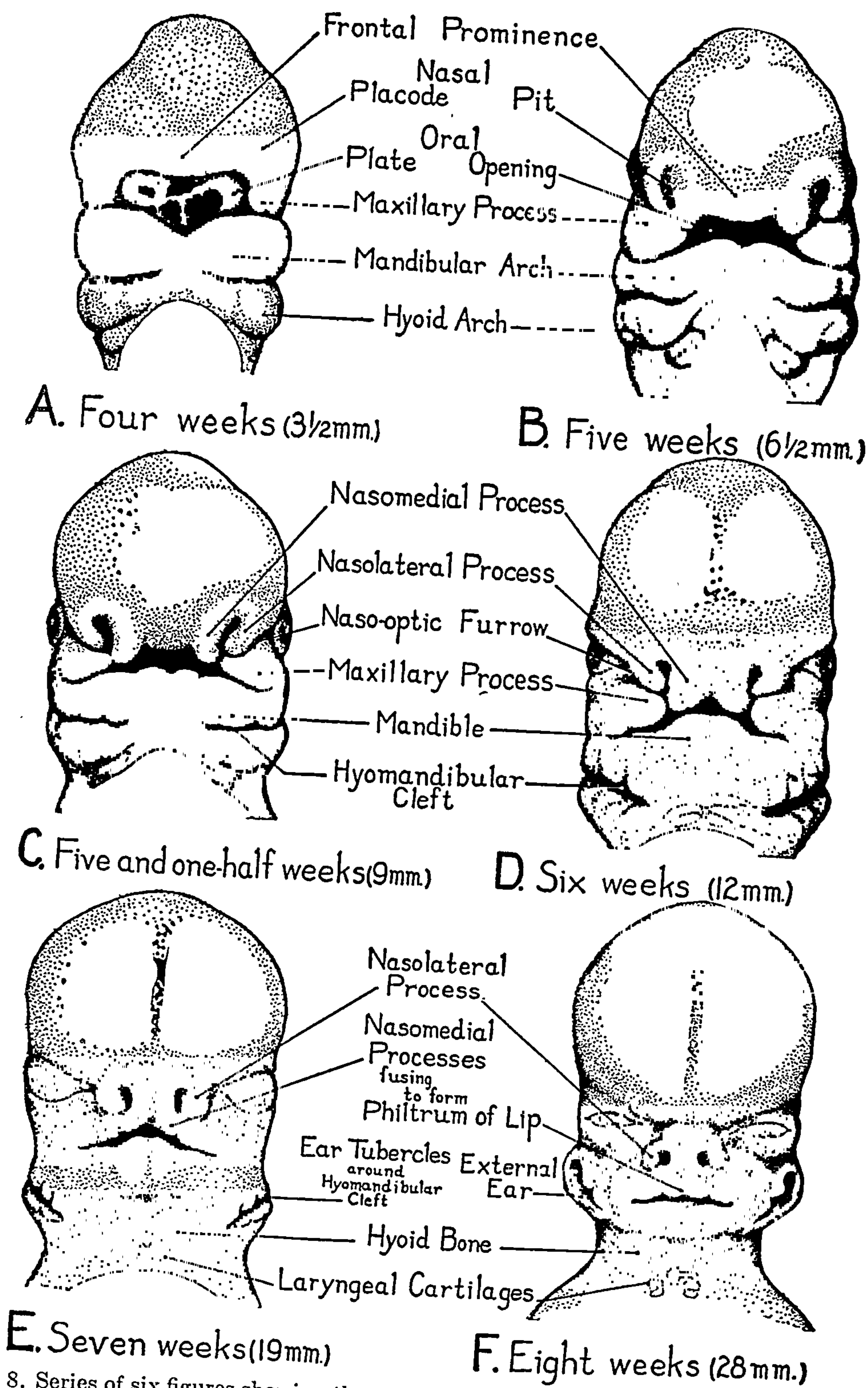


FIG. 8. Series of six figures showing the early development of the facial region. (Courtesy of B. M. Pattern.) From Morris' Human Anatomy, 11th ed., Edited by J. Parsons Schaeffer. New York, Toronto: Blakiston Company, 1953.

The cartilage cells appeared like those of any well-fixed and stained living rib cartilage. The nuclei were intact and the cells only moderately retracted from the walls of the lacunae. The chondrocytes were probably viable three and a half years after transfer in the tissues of another human.

A fresh cartilage homograft removed four years after transplantation and sectioned in the fresh unfixed state appeared to contain viable chondrocytes.

In general, those cells which retain their capacity to divide continuously do not age. Thus mitosis appears to be a constant rejuvenizing action, which retards the process of senescence. Such continuously-mitosing cells in tissue cultures are potentially immortal. Conversely, well-differentiated cells such as nerve cells, which lose the ability to reproduce in early infancy, undergo a progressive sequence of aging and death.

Senescence appears to be caused partly by changes in the fluid medium in which cells live; for most cells this means the interstitial fluid rather than the blood. Studies by Carrel (5) have demonstrated that blood plasma contains both growth-promoting substances and inhibitory substances. The latter increase with age and reach a high concentration in old age. These inhibitory substances may be a factor in the causation of senescence. It also seems probable that the life span of the cells which undergo senescence is partly determined by heredity.

CHEMICAL REACTIONS

The cell resembles a small chemical factory which is equipped to accomplish synthesis and breakdown of various substances at the level of body temperature. Chemists can carry out many of these same reactions in the laboratory but under conditions of high pressures and high temperatures which are not present in the human body. The tissue cell accomplishes these chemical reactions through the action of enzymes, substances capable of affecting such reactions at body temperature.

It is now understood that the presence of certain trace substances is necessary for the building up of enzymes, which are constantly being destroyed by the chemical reaction in which they are involved. The presence of certain vitamins is essential for the effectiveness of the enzymes (the enzyme-vitamin complex), and hormones in turn play an important part in regulation and control.

Essential cell activity may not only be interrupted by the absence or deficiency of the substances enumerated above but it may also be adversely affected when they are present in excessive amounts. Certain substances therefore are produced by the body to serve as inhibitors or neutralizers so that a normal rhythm of activity in the factory cell may continue. Alteration in any essential link of this vital chain of factors may cause the living tissue cell to age and eventually die just as surely as taking away its raw materials—the food substances—which the enzymes, vitamins, and hormones affect.

Substances such as the raw materials, or food, water, hormones, vitamins, and the trace elements necessary for the action of the enzymes, can be controlled by the surgeon to a large extent. All factors are essential for the survival of free tissue grafts, which are composed of living cells and matrix. The ultimate fate of the transplant usually depends on the survival of the living cells in the grafts. A circulating blood supply is necessary for all vascular tissues, but this circulating blood must provide interstitial fluid which contains the substances required for cell life and cell activity.

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CHAPTER 5

Intercellular Dead Substances Surrounding Tissue Cells

The process of cell division initiated by the fertilized ovum is a simple increase in the number of cells up until the blastula has been formed. A group of cells known as the organizer,[1] which is located in the dorsal lip of the blastopore, then assumes control of the cell division and causes a grouping of cells into the three germ layers—ectoderm, mesoderm and endoderm.

The formation of three germ layers represents a sorting out of tissue cells to provide a proper and efficient division of labor for building the human body and to provide for its future servicing and maintenance.

The nuclei of cells in the three primary germ layers undergo little change but the cytoplasm differentiates and becomes specialized to such an extent that the mature progeny have little resemblance to the original. Examples of these differences are seen in the varied structure and activity of skeletal muscle cells, fibroblasts, nerve cells, and fat cells. Regardless of these differences due to changes in the cytoplasm the cells retain their basic similarity; all have a nucleus contained in the nuclear membrane, and a plasma or cell membrane surrounding the specialized cytoplasm.

The non-living intercellular substance surrounding cells is believed to be produced and maintained by the activity of the living cells, or in association with them. This activity may occur as a budding off of cytoplasm to form intercellular collagenous fibers as in the construction of a tendon, or the cell may appear merely to stimulate and maintain the building operation as in bone.[2] The cell nucleus, although it appears to be resting, possibly acts in a supervisory capacity, but this is not definitely known.

ORGANIZATION OF CELLS

The cells in the three primary germ layers undergo further differentiation, resulting eventually in highly specialized cells with definite functions. Thus, cartilage cells produce cartilage matrix; bone cell elements influence the deposition of calcified intercellular substance in which they are later surrounded; tendon and fascia cells throw off cytoplasmic buds which form collagenous fibers; and epidermal cells, with little intercellular substance, reproduce endlessly to

[1] See Chapter 4, (Spermann and Mangold).

[2] There is disagreement regarding the cellular agency responsible for new bone formation (osteoblast or undifferentiated connective-tissue cell) and the cell responsible for the manufacture of elastic fibers is also unknown. It is important to note, however, that intercellular substances are not ordinarily formed when living cells are absent.

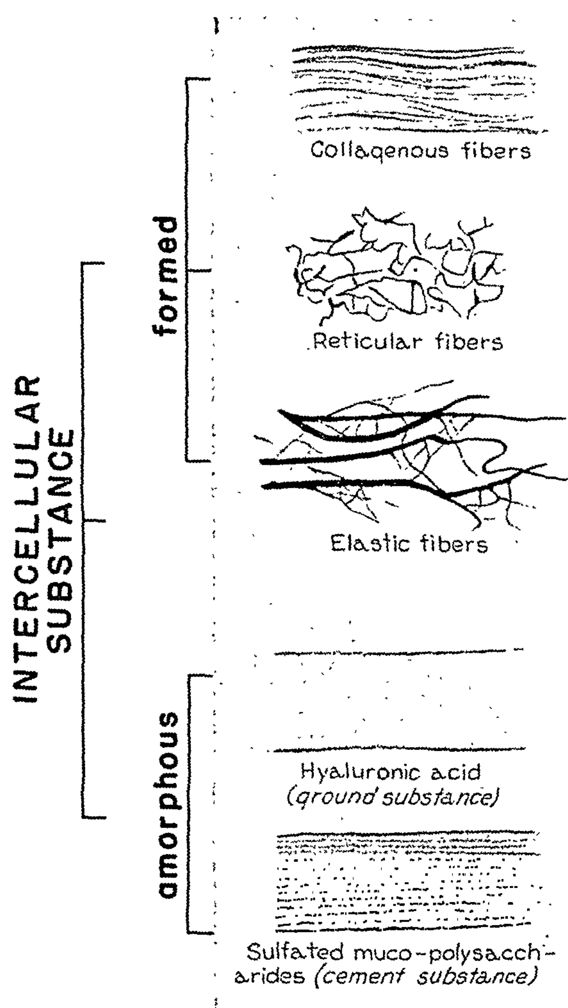


Fig. 9. Non-living matrix between cells. (Modified drawing after Ham, 1950.)

provide a protective horny layer on the body surface.

Some cells retain their embryonal or more primitive characteristics, and it is believed that these cells can do a number of different things which are essential for the servicing and maintenance of the body when the occasion arises.

Many cytologists believe that ordinary fibroblasts in fat and other connective tissues are specialized cells incapable of changing into other types of connective-tissue cells, but investigators have noted true bone formation in and around preserved cartilage grafts buried in human abdominal fat. This true bone formation, which has also been observed in living autogenous human cartilage grafts buried in abdominal fat, must have been produced by fibroblasts in the fat stroma, unless one assumes that undifferentiated fibroblasts capable of bone formation migrated to or were present in the recipient area. There was no evidence that the living cartilage cells in the autogenous graft took part in the bone formation.

SPECIALIZATION OF TISSUE

Thus, through specialization in the cytoplasm of cells and by the elaboration or controlled deposition of different intercellular substances, various tissues and organs are constructed. In muscle, fatty tissue, and nerve tissue, these alterations in the cytoplasm are important factors, whereas in bone, cartilage, and tendon the dead intercellular substance dominates the scene and provides important qualities for these tissues.

One must bear in mind, however, that the small modified fibroblasts in fascia, cartilage, and tendon are the living parenchymal cells which may maintain the important matrix. If the cells die and are not replaced by living cells, the matrix of these tissues tends to disintegrate.

The development of specialized tissues from the ectoderm, mesoderm and endoderm is not however, a mere matter of cell division and cell differentiation. As pointed out by Medawar (1), "the tactics of embryonic development and of regeneration are a matter of movement of cell substances, cells and cell groups." Cell division and synthesis are called upon to play their part but these are properly timed with the rhythms of mass movement. The orderly and controlled development of highly specialized human tissues from a mass of undifferentiated cells represents a cooperative effort of tremendous magnitude. During the process "a division of labor" occurs among cells, some having one task and others having another. In this way there are developed specialized tissues and organs made up of many different kinds of cells, which have considerable variation in size, shape, and function.

Intercellular substances, which may be

fibrous or amorphous jellies, are the dead parts between cells. This intercellular substance serves to hold the body together, providing rigid strength as in calcified bone and elasticity as in the gelatinous matrix of cartilage, whereas in other areas it supports the cells in a loose and movable mesh.

Since intercellular substances are usually interposed between capillaries and the cells which they nourish, all, regardless of their apparent density, must permit diffusion of substances from capillaries to cells and vice versa. Amorphous intercellular substances such as gels or sols usually permit better diffusion than fibrous substances.

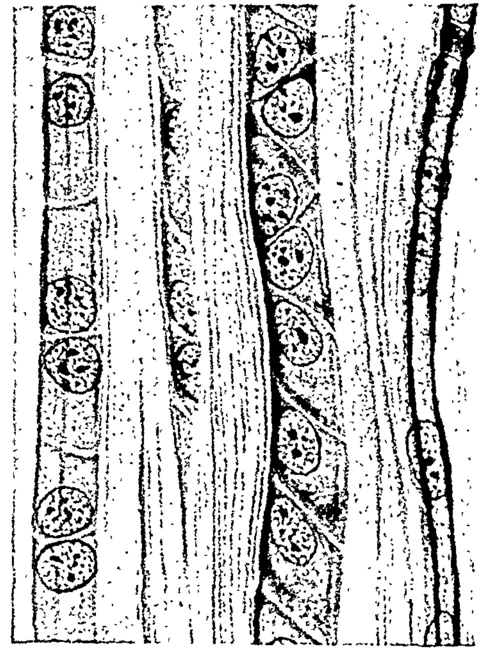


FIG. 10. Freshly teased tendon of the tail of a rat, stained with methylene blue. The rows of tendon cells run between the collagenous bundles. 520X. From A Textbook of Histology 6th ed., Alexander A. Maximow and William Bloom. Philadelphia & London: W. B. Saunders Co., 1949.

COLLAGENOUS FIBERS

Collagenous fibers, which possess great tensile strength, on boiling become collagen; this in the hydrate phase is gelatin. The fibers appear to have longitudinal striations due to the fact that each fiber is composed of numerous fibrils running in the longitudinal direction. Electron-microscopic examination by Schmidt and his coworkers (2) has shown that the individual fibrils have a cross-striated appearance, suggesting that they are composed of alternating bands of material. The plane of cleavage of fibrils, however, is longitudinal, which suggests to Schmidt the presence of protofibrils in the fibril.

The manner in which fibroblasts lead to the formation of collagenous fibers has been a source of controversy for many years. Some held that the fibers are deposited in the intercellular substance as a sort of precipitation reaction due to an enzymic action controlled by the fibroblast, while others believed that the fibers are differentiated within the cytoplasm of the fibroblast, to become subsequently extruded.

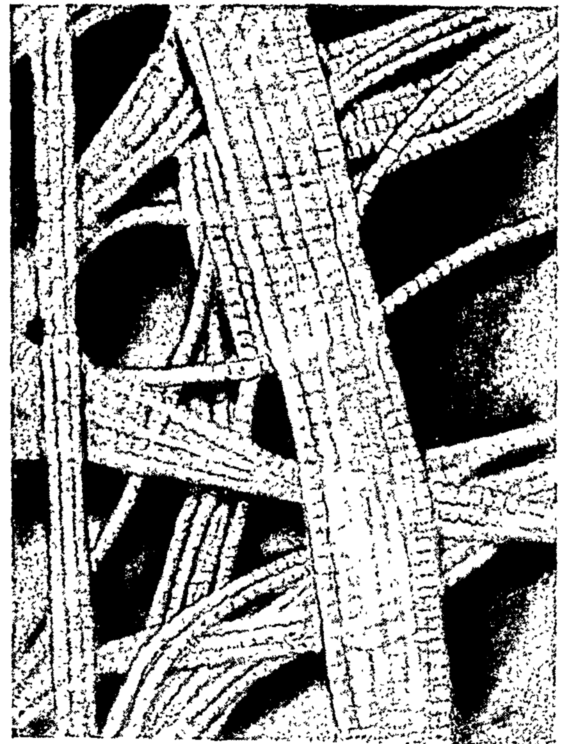


FIG. 11. Drawing of collagenous fibers from fresh tendon of rat's tail. Viewed with electron microscope. X26,400. (Redrawn from Schmidt, et al.)

Le Gros Clark (3) suggests that the question has been settled by Stearns (4), who observed, with the transparent chamber, the fibroblasts in healing wounds in rabbit's

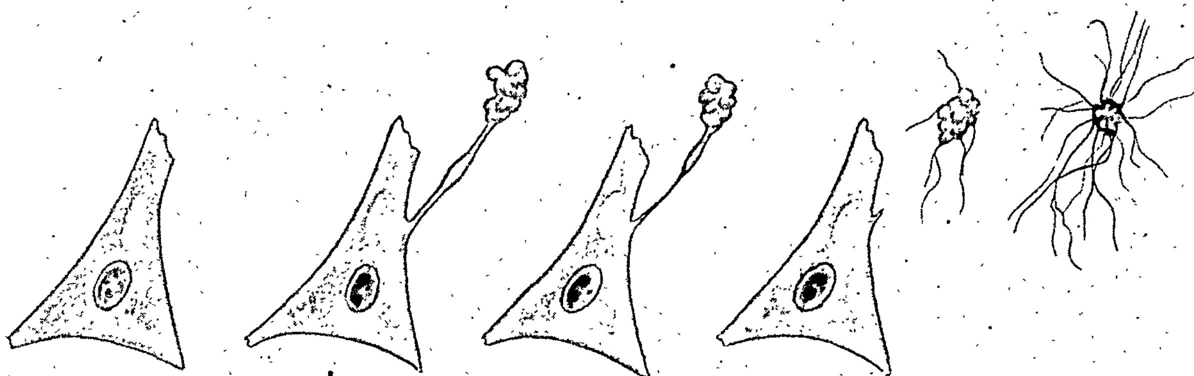


FIG. 12. Diagram showing how collagenous fibers are formed by fibroblasts. (Redrawn after Clark.) From a paper by M. L. Stearns, Am. J. Anat. 67: 55, 1940.

ears. Stearns showed that during wound healing the fibroblasts throw off small masses of cytoplasmic materials which, after they are completely free of the cells, give rise to the formation of collagenous fibers. The process is a very rapid one, and in three to four hours each fibroblast is surrounded by a dense network; in forty-eight hours the network may become so dense that the cells are completely obscured. The rapidity of the process may account for the fact that it is not observed more often. No evidence was found that fibrin could differentiate into collagenous fibers (3). Tension appears to be a mechanical factor in stimulating the production of collagenous fibers and also in determining their direction.

Collagenous fiber formation is retarded in the absence of vitamin C (ascorbic acid). These fibers make up the bulk of intercellular substance in fascia, tendon, and dermis, are present in bone and cartilage, and form a supporting structure for fat cells, muscle cells, and the bundles of axons in peripheral nerves.

How long collagenous fibers retain the physical properties on which their usefulness depends is a mystery. Although we now believe that the fibroblast produces the fibers by throwing off its small bud into the intercellular space, we do not know how long a collagenous fiber lasts. Is it replaced at intervals or does it last until the death of the individual? Certainly it can be replaced abundantly when there is a loss of tissue or a need for its presence in wound healing.

The overproduction of collagenous fibers and homogeneous matrix in hypertrophied scars and keloids is a cause of great frustration among plastic surgeons. Roentgen-ray or radium therapy applied over a fresh wound after a keloid has been excised apparently retards excessive activity of the fibroblasts, so a smaller scar results than when excision is not followed by such therapy. Skin grafts applied over an area from which a keloid has been removed do not show keloid formation beneath. Keloid will develop, however, around the periphery where the graft joins the recipient skin, unless this is exposed to irradiation. Members of the Negro race tend toward keloid formation, as do many young children and some infants. Children often outgrow the tendency before or after puberty. Patients in whom keloid did not form previously may develop it after a severe burn. Administration of cortisone or ACTH and hyaluronidase does not appear to have helped or prevented re-formation of keloid in susceptible patients. To my knowledge no one has tried vitamin C deficiency after excision for rather obvious reasons.

Pullinger and Pirie (5) in 1942 discovered that implantation of collagen results in chronic inflammation. They suggested that the breakdown of collagenous fibers may cause some of the chronic inflammatory lesions frequently seen in elderly patients.[3]

[3] Strips of fascia lata and sliced tendon grafts are often transplanted beneath the facial skin as

ELASTIC FIBERS

lastic fibers, which are much less numer- than collagenous fibers, appear as rather :ate homogeneous threads; these branch uently, forming a sort of loose network. lastic membrane, such as the ligamentum us of the spinal cord, where numerous tic fibers are close together, they appear ; yellow in color. According to Ham (6) , consist of the protein elastin, which of body proteins is probably the most re- ınt to chemical change. The elastic fibers :he arteries in Egyptian mummies are ın sufficiently well preserved so that con- ;ions can be drawn about arterial diseases he people of that era.

lastic fibers appear homogeneous even ler electron-microscopic examination and not seem to be composed of fibrils, as are agenous fibers. As the epithet elastic im- s, elastic fibers when stretched and sud- ly released tend to snap back like rubber ıds. They undoubtedly provide the tissues which they reside with an important and icient degree of elasticity.

Elastic fibers are fairly abundant in the mis[4] of the skin, where they form a loose work with the greater mass and number collagenous fibers. Elastic fibers are re- ted to be present in tendon, fascia, and ; stroma of nerve, muscle, and fat as well

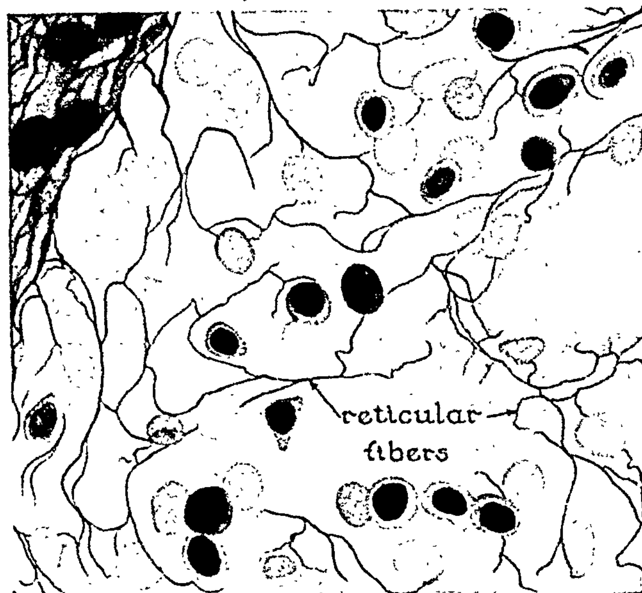


FIG. 13. Drawing of a section of spleen stained by the Bielschowsky technique for reticular fibers (high-power). By this method, silver is precipitated on the reticular fibers, which makes them stand out as fine black lines. From Histology, Arthur Worth Ham, M. B. Philadelphia: J. B. Lippincott Co., 1950.

as in the cartilage of the ear, where they occur in great numbers.

According to Le Gros Clark (3) and Cowdry (7) the developmental origin of elastic fibers is still obscure. Clark states that they appear to be constructed from the alignment of refractile granules laid down in the intercellular matrix by some unknown agency. Probably they are not formed by the fibroblast.

There is no evidence that new elastic fibers can be formed when they have been destroyed, and it seems probable that the original fibers last through the individual's life time.

RETICULAR FIBERS

These fibers, as the name suggests, form a reticulum or mesh and occur most abundantly in organs where they provide a yielding support for the cells. They also form an important part of the lymph glands and can be identified in the areolar tissue beneath the skin.

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ogenous grafts to support a paralyzed muscle. th tendon and fascia are abundantly supplied h collagenous fibers, and a large number of se fibers are necessarily injured during opera- n. These tendon and fascia grafts do not give to an inflammatory reaction in the host tissues humans other than the rather mild initial reac- n caused by transplanting any autograft. When cia grafts are removed one year later no cellular ivity is noted around them; they have retained ir structure as fascia grossly and microscopi- ly.

[4] I have identified elastic fibers in the dermis of ree autogenous skin graft 40 years after trans- ıntation. The elastic fibers in a free autogenous r cartilage were identified four years after trans- antation of the cartilage.

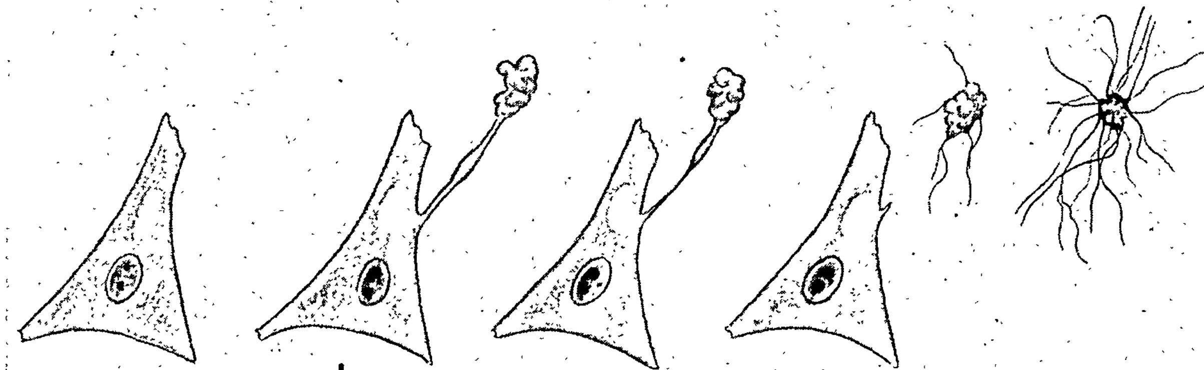


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[3] Strips of fascia lata and sliced tendon grafts are often transplanted beneath the facial skin as

separate farther apart; this increases the size of the intercellular spaces between cells.

In discussing the intercellular substances and their aging, Ham (13) notes that the ratio of amorphous to fibrous intercellular substances becomes lower throughout life; tissues of infants having a large amount of amorphous material and those of aged individuals having a large amount of fibrous intercellular material. Thus sutures, which hold rather well in older persons, tear out or cut through the skin of newborn infants. Similarly, meats from older animals are tough due to the large content of fibrous intercellular substance and must be cooked for longer periods of time to convert the collagenous fibers into gelatin, which makes the meat more tender. The flesh of young animals containing less collagen is rendered tender when it is cooked a much shorter period of time.

The shift from amorphous to fibrous intercellular substance in aging may adversely affect diffusion from capillaries to cells, and vice versa.

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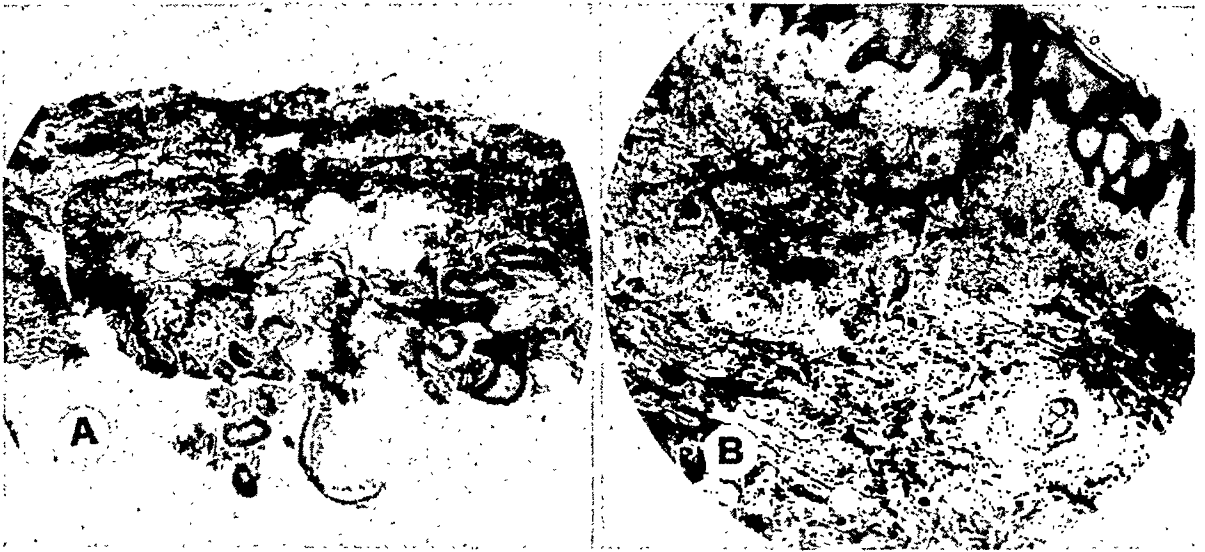


FIG. 14. A. Full thickness skin graft from arm to eyelid. Graft was removed 40 years after transplantation. Special elastic stain demonstrates that elastic fibers in the dermis are present. The sebaceous glands, sweat glands, and epidermis also survived transplantation, and collagenous fibers and fibroblasts in the dermis appeared entirely normal as shown in other sections. (Author's series.)

B. Full thickness skin graft transplanted from the arm to the eyelid and removed after 40 years. The epidermis, hairs, and glands have all survived with their living parenchymal cells and orderly structure. ×100.

The relationship between reticular and collagenous fibers is still a controversial issue, some believing that they are fundamentally different and others that they are quite similar. Maximow (8) held the view that they are similar, and showed instances of anatomical continuity between the two. In connective tissue Wolfe and his colleagues (9) demonstrated a gradual transformation of reticulum into collagen in the process of aging, while Ham (10) states that the difference between reticular fibers and collagenous fibers is mainly a difference in size. Le Gros Clark (11) regards reticular fibers as immature collagenous fibers and notes that in the process of aging the delicate reticular basketwork tends to be converted into layers of inelastic collagenous fibers. Conversion of the subcutaneous reticular fibers beneath the facial skin into inelastic collagenous fibers may be a factor in causing cutaneous sagging in elderly persons.

AMORPHOUS INTERCELLULAR SUBSTANCE

The fibrous types of intercellular substance are usually surrounded by the amorphous variety. Thus collagenous fibers in bone and hyaline cartilage are embedded in a ground or cement substance which obscures them. In hyaline cartilage the ground substance has the same optic index as the fibers—hence the matrix appears homogeneous. This illusion can be demonstrated by dropping a glass cover slide into a glass of water and noting that it seems to disappear.

The amorphous intercellular material consists of cement substances (sulfated mucopolysaccharides) and a ground substance (hyaluronic acid). Hyaluronic acid has a cohesive quality, which holds the tissue cells together. An enzyme hyaluronidase (12) reduces the viscosity of hyaluronic acid and, in separating its molecules, produces the spreading factor which allows the cells to

separate farther apart; this increases the size of the intercellular spaces between cells.

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CHAPTER 6

Structure and Viability of Cells

During the nineteenth century histological investigation was characterized by the desire for a better understanding of the origin and function of the parts of animals and plants as evidenced by minute structure. The histologists of a hundred years ago studied the membranes and scrapings of thick tissues because most tissues and organs are too thick to be examined directly with the microscope. The scrapings and teasings disrupted normal relations and the cells soon died. Considering the imperfect microscopes and the limited number of reagents available, it is astonishing that these early investigators were able to make as many accurate observations as they did.

METHODS OF STUDY

During the next fifty years the study of living and surviving cells was replaced to a large extent by interest in structural details rendered visible in dead cells by methods for preservation of tissues, cutting them in thin shavings and staining them with various dyes. During this period a large amount of accurate data about the modes of reproduction, methods of growth, differentiation, and death of cells was accumulated.

Bloom (1) notes that a new era began about the turn of the century, characterized by attempts at interpretation of structure in terms of function, and by renewed interest in correlating appearances of living and dead material. As one result of this study, a number of investigators began to look at *all sectioned material as artifacts* and to accept as true only what could be seen in living cells.

This view was soon modified, because some of the so-called artifacts of fixation have been shown to be important components of living cells. On the whole, however, the skeptical period has caused the histologist to realize that *he must use all methods of investigation, that even all of them together are insufficient and that new ones must be developed.*

Out of this period several significant new methods of study developed—such as tissue culture, transplantation of free grafts to various host tissues including the anterior chamber of the eye, the use of transparent windows in the ears of rabbits and mice, and micromanipulation devices.

Today, newer techniques in histology, recognizing the advances in physiology and biochemistry, are concerned with a more precise description of structure in terms of chemical and biochemical composition and biochemical activities, and with the development of new physical instruments capable of revealing finer structural details. Micromanipulation and microtransplantations of nuclei, centrioles and other cell structures

into tissue culture and into other cells are among the important recent approaches to a better understanding of cell physiology.

Plastic surgeons, who use free grafts to correct deformities, are becoming aware of the importance of the cells in their tissue transplants. Others have utilized gland, organ and blood vessel transplants as homografts with varying degrees of success. This has created considerable interest in the behavior of various tissues under the conditions of free transplantation and has, of necessity, forced the surgeon to consult the zoologist, biochemist, histo-anatomist, and immunologist for aid in understanding the behavior of his tissue transplants. A recent meeting under the auspices of the New York Academy of Science on "homografting" was attended by pure scientists and clinicians from all over the world. This meeting resulted in a "pooling of knowledge," which may have a profound effect on the progress and development of clinical medicine, and clinical histology.[1]

TISSUE CULTURE

The most difficult and frustrating problem in evaluating the behavior of free tissue grafts is to determine accurately whether the cells have remained viable or are dead structures, which take the various dyes and demonstrate normal-appearing cell architecture.

One of the methods which permits us to observe the cells not only in a state of mere survival but under more favorable conditions, and also to follow their development, is *tissue culture*, originated by Harrison (2) and developed by Carrel (3).

Tissue culture, which was formerly used largely for embryonal tissues, has more recently been applied as a means for determining the viability and growth properties of the cells in animal and human tissues.

[1] It appears that a new science, clinical histology, is in process of developing.

Strangely enough, tissue culture has not been employed as a means of determining the viability of the cells in any of the human transplants discussed in this book. It has not been used by me, because it is not available locally, and tissue culture departments in the medical colleges and research institutes in New York City are too occupied with *in vitro* studies of cancer cells and the like to concern themselves with free human tissue grafts. If a second edition of this volume is ever published, it will include the results of tissue culture studies on all free grafts in humans.

The technique of tissue culture consists in transplanting small portions of various tissues in a suitable medium where the cells can adapt themselves and grow in an autonomous form. Undoubtedly this would be the most accurate and final way to determine whether the graft cells have survived or have failed to survive, provided a tissue growth is obtained. If growth does not occur, the possibility would still remain that the graft cells were viable but were not capable of growth in the media. Different adult human tissues vary in their ability to grow in tissue culture.

When cells grow in tissue culture they spread over the coagulum of plasma and pass out from the transplant to form a "zone of growth," which permits vital observation of these cells. The field of tissue culture and its application to a study of the fate of cells in free grafts should develop rapidly because it is an important approach to further knowledge of cell and graft behavior.

FIXATION AND STAINING OF CELLS

While the examination of living cells reveals few morphological details, with methods of fixation and coloration the aspect of the cells is more complex and varied. In addition to the structures that are observed *in vivo*, which are more or less

modified by fixation, other structures are found which were not apparent before, due to the similarity of their index of refraction with that of the rest of the cell.

Bloom (4) notes that it is the organization inherent in protoplasm which confers the attributes of life to the complex chemical machinery. To preserve the structure for microscopic examination destroys the dynamic organization and to a greater or less extent removes some of the structure and distorts the remainder. That we can recognize the preserved material at all is largely due to certain properties of the proteins which are universally present. The first step in "fixation" of protoplasm involves rendering the proteins insoluble by precipitating them.

Fixation is essentially a method of preserving the morphology and chemical composition of the cell (5). The object of the fixation is to bring about the death of the cell in such a manner that the structure which the living cell possesses is conserved with the minimal addition of artifacts. Some methods, at the same time, attempt to maintain the chemical composition of the cell as intact as possible. Fixation therefore has two fundamental aspects, one cytomorphological and the other cytochemical. According to de Robertis *et al.* (6), when a piece of tissue is submerged in a fixing liquid, the death of the cells does not occur in an instantaneous manner. The fixative penetrates into the piece by diffusion from the periphery toward the center in such a way that the most external cells are fixed more rapidly and better than the central ones. For this reason in every fixed tissue there is always a "gradient of fixation," which depends upon the "penetrability" of the fixative, its progressive dilution with the liquid of the cells, and the postmortem alterations which occur in the cells due to anoxia, changes in the concentration of hydrogen ions, and enzymotic action. The rapidity with which the fixative penetrates does not appear to depend so much on its coefficient of diffusibility as on the protein barrier impeding further passage of the fixative.

Fixatives also produce currents in cells and these may displace the soluble components. Besides displacing the soluble substances, fixatives extract with greater or less intensity. Thus, the electrolytes, soluble carbohydrates, and even lipids may leave the cells by the action of the fixatives.

Fixation and later treatment produce a shrinkage of the tissue and this is important in interpreting the cytological images in fixed tissues. *"One should always remember that the volume of fixed cells is less than that which they had in the living state."*

The interpretation of the structural aspects of the cells in fixed tissues should therefore be made with caution *but not with excessive skepticism.* Beside the examination of living cells the examination of fixed and stained cells is essential to a better understanding of cellular structure. *The two methods are complimentary and one does not exclude the other.*

FIXATION BY FREEZING AND DRYING

It appears, therefore, that although fixation often permits the preservation of the cell in its true morphological aspect, it may produce considerable chemical modification.

A technique devised by Altmann (7) and brought to practical use by Gersh (8) permits us to investigate the morphological structure and the distribution of chemical components with a minimum of modifications.

De Robertis (9) describes the technique as follows: Drying can be accomplished by reducing the partial pressure of water vapor in the atmosphere surrounding the frozen tissue to a point below the vapor pressure of water at the drying temperature. When

this is achieved at low drying temperature, the water passes off from the solid phase in the frozen tissues directly into a gaseous state (sublimation) without any intervening liquid phase which might distort the cell. In this way there is produced a progressive dehydration which reaches practically to the extraction of all the water contained in the tissues except for a residue which is firmly bound.

The advantages of this method are obvious. It does not produce shrinkage of the tissue; the fixation is more or less homogeneous in the entire thickness of the piece; there is no extraction of soluble substances; the chemical composition is maintained practically without change; and the structure, in general, is preserved with very few modifications produced by ice crystals.

The rapidity of fixation permits one to trap and preserve cells at critical moments of their function such as at the moment when kidney cells are excreting colored material or other substances (Gersh) or when thyroid cells are extruding colloid droplets into the follicular cavity (10).

Cecil Taylor[2] has been concerned with the storing of living tissues at temperatures below freezing for their ultimate use as grafts. In rats and mice the cells of tissue are destroyed when frozen without pretreatment. *When freezing, however, is preceded by treatment of the tissue with a solution of glycerol or ethylene glycol, there is considerable survival;* the viability being judged by growth of the cells in tissue culture, and by the cytological appearance of cells and growth of hair when the tissue is transplanted back into the original donor animal. The cytological effect of these different methods of pretreatment and freezing are being studied by phase microscopy and motion pictures of the living cells. *Application of these findings is now being made with skin grafts on human volunteers* by Rogers.

The freezing-drying technique of Altmann-Gersh should be considered as an intermediary between the examination of fresh and fixed tissues. Frozen-dried cartilage and bone banks have been developed at Bethesda, Maryland, by the Armed Services. These frozen-dried grafts are stored in a vacuum (bottle) and twenty-four hours before they are to be used, normal saline is introduced into the bottle so that the desiccated graft will absorb moisture and in general regain its original weight and appearance.

VITAL AND SUPRAVITAL DYES

According to Ham (11), both vital and supravital staining depend on the interaction of vital activity and a dye. *"Neither vital nor supravital stains will produce their characteristic effects if they are applied to dead cells."*

Vital staining is accomplished by injecting into living animals, usually intravenously, certain dyes of a colloidal nature (trypan blue) or certain metals in a colloidal state (colloidal silver) or fine particulate matter (suspension of fine carbon particles as in India ink). The procedure is not intended to kill the animal or injure it to any great extent. The colloidal particles so injected tend to be removed from the circulation by a family or system of cells in the body known as the reticulo-endothelial or macrophage system. The cells of this system are phagocytic and possess the ability to incor-

[2] Preliminary report on recent advances in transplantation studies. The Plastic Surgery unit, under the direction of Dr. John Marquis Converse, Department of Surgery, New York University, College of Medicine: Transpl. Bull., 1: 154, 1954; cited with permission of the investigators (John Marquis Converse, M.D.; Mario Gaudino, M. D., Ph.D.; A. Cecil Taylor, Ph.D.; Blair O. Rogers, M. D., and Jerome W. Lehrfeld, M.S.)

These preliminary reports of indefinitive experimental work are not ordinarily included in this volume.

porate the colloidal particles of the vital stain into their cytoplasm and become colored by the dye particles which they accumulate.

Supravital staining (11), while requiring living cells to be effective, is not usually accomplished by injecting dyes into the circulation of living animals but rather by 1) injecting dyes into the blood vessels of animals immediately after they have been killed and while the cells to be stained are still surviving, or 2) applying dyes to freshly removed pieces of tissue in which the cells are still alive.

Workers in the Peer Clinic have used dilute solutions of supravital dyes to stain the cells in fresh shavings of autogenous human cartilage grafts, and have also applied the supravital dyes to fresh control cartilage shavings and to preserved cartilage with dead cells. The observations made on a large number of autogenous grafts in this manner indicate that *the cartilage cells in human autografts survive transplantation as living chondrocytes. Cartilage, because of its physical properties, is unique among all other tissues in that it can be cut with the microtome into the thin shavings without the necessity of freezing the tissue or of using fixative agents*, both of which alter the structure and chemical properties of the cells. Keloids also may be examined in the fresh state to some extent. With this method there is a complete absence of shrinkage and the chemical composition is maintained practically without change.

The technique is as follows: Immerse the fresh autogenous graft in melted paraffin which is just about to solidify and immediately place the paraffin enclosed graft in ice water, which hastens solidification and prevents heat damage to the centrally located cells in the cartilage piece. When solidification has occurred, the paraffin block is placed in a microtome and sections are made, which are caught in Ringer's solution or normal saline to prevent drying and death of the cartilage cells. The sections are then placed on a slide, covered with a few drops of normal saline and examined microscopically. The chondrocytes will be seen clearly in their normal state, with reticular cytoplasm and large well-demarcated nuclei completely filling the lacunae. *It appears that these are living cartilage cells because they are exactly like the cells seen in sections of fresh control cartilage.* As the saline solution evaporates the centrally located cartilage cells will be seen to retract from the walls of their lacunae and lose all form of normal cell structure, thus resembling the dead chondrocytes in fixed and stained cartilage sections. One may also apply dilute solutions of supravital dyes to fresh sections of an homogenous human cartilage graft and observe that the chondrocytes take the dyes as living cells, thus indicating that they have survived and are viable.

TRANSPARENT WINDOWS

The transparent chamber technique was modified by Algire and Legallais (12) for the study of tumor transplants and was first applied for a study of autogenous and homogenous skin grafts in mice by Herbert Conway *et al.* (13) in 1951. Doyle Joslin (14) in 1952 further modified the transparent chamber as devised by Algire and Legallais. This modified chamber has been used by Conway and his associates to study the development of the circulation in autogenous and homogenous skin grafts.

Clear observations may be made through transparent windows which can be inserted by surgical procedures into some part of the body. Sandison (15) invented the method in 1924 by cutting holes in rabbits' ears and using the borders of these as window frames. By placing the rabbit ear on the stage of a microscope with the window directly

under the objective, he was able to observe the growth and movement of the living cells.

This is a valuable method for the study of the behavior of tissues and its use will undoubtedly be extended to determine the fate of all types of free transplants. (Conway is currently using the transparent chamber technique for the study of bone grafts (16).)

The method of approach, however, has certain deficiencies and again demonstrates the necessity for the use of all available methods of investigation, since all of them together are often insufficient to establish definite facts which cannot be questioned.

Taylor and Lehrfeld (17), for instance, using special lighting, made a direct study of the development of the circulation in homogenous skin grafts after they had been transplanted to their host site. They demonstrated circulating blood in the grafts in about three days after transplantation, whereas Conway *et al.* using the transparent chamber technique, were unable to observe circulatory blood in skin homografts.

THE PHASE MICROSCOPE

The light microscope has also been improved by the development of phase-contrast microscopy. Many of the minute constituents of tissue are quite similar in respect to optical density. For this reason they transmit and refract light almost equally, and when fresh unstained tissue is examined with the ordinary microscope, the particles do not stand out in contrast with one another. Often they can barely be seen as individual structures. This poor image led to the general use of stains which more or less selectively color the different small tissue constituents and thus permit them to be visualized as such.

The phase-contrast microscope permits the minute structures which differ from one another in optical density only slightly, to be brought into sufficient contrast to permit their successful study in fresh unstained tissues.

According to Ham (18), the phase microscope makes it possible to study either living or fresh unstained tissue much more effectively than hitherto. In some instances, it is useful for bringing out fine detail in stained sections.

The electron microscope, dissection of cells under the microscope by manipulating instruments (see Kopac, Volume II), and the ultracentrifuge are additional agencies which are of great value in determining the structure, chemical composition, and physiology of cells. None of these techniques has as yet been applied to human tissue grafts.

THE DISSECTING MICROSCOPE

Taylor and Lehrfeld (17) have examined skin autografts and homografts *in situ* in rats, mice, and rabbits through the dissecting microscope. They noted that the circulation of blood within the capillaries of grafts is similar in both autografts and homografts, and the time of breakdown of circulation (at about the seventh or eighth day) is consistent in rats, mice, and rabbits. The development of a circulation in both autografts and homografts is rather strong evidence that the cells in the grafts are viable, and this direct observation of skin grafts through the dissecting microscope is a valuable contribution to our knowledge of the subject.

The gross appearance and histological sequence of events in the temporary survival of skin homografts in human volunteers were studied by Rogers (19). He confirmed Medawar's observation in rabbits that a second crop of homotransplants from the same donor to the same recipient did disintegrate at a more rapid rate than those of the first crop. A marked eosinophilia, both systemic and local, was observed in full thickness skin homografts.

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CHAPTER 7

The Parenchymal Cell in Free Tissue Grafts

The parenchymal cell is the essential or specialized part of a tissue or organ as distinguished from the supporting connective tissue. It would be well for every surgeon to have a clear conception of the parenchymal cell and of the factors which affect its survival in manipulated and transplanted tissues, because the viability or death of this cell often determines the fate of the tissues.

Experimental work and clinical observation indicate that survival of the parenchymal cells is associated with retention of the particular tissue structure in living autogenous grafts of cartilage, fascia, tendon, fat, skin, and certain bone grafts.

In free skeletal muscle grafts with detached nerve and blood supply the muscle cells always die. Dead bone in contact with living bone may be replaced by "creeping substitution" from the living bone with which the dead bone makes contact. Free nerve grafts are in a special category.

It is important, therefore, to identify and understand the requirements of this parenchymal cell, the survival of which is essential for the success of at least six important types of free autogenous grafts and should be properly evaluated for intelligent handling of all grafts, and for all tissue manipulation.

MATRIX

The living parenchymal cell is not only responsible for or associated with the retention of *an intercellular substance;* it also appears to determine generally that this dead matrix remain as the *same kind of intercellular substance* in six types of autogenous tissue grafts. In this manner the matrix of hyaline cartilage remains hyalin, the matrix of elastic ear cartilage remains as elastic substance, the collagenous fibrous matrix of tendon, fascia and dermis remain of the same character, as does the small amount of intercellular material surrounding grafted epidermal and fat cells and the calcified matrix of certain bone grafts in soft tissue sites.

Free autogenous human grafts in which the parenchymal cells have been intentionally killed before transfer do not tend to retain their normal matrix structure. Thus, autogenous cartilage grafts with dead cells tend to be gradually absorbed and replaced by fibrous connective tissue or bone; autogenous tendon and fascia grafts with dead fibroblasts do not retain their

same structure.[1] Autogenous septal bone grafts with dead cells in soft tissue sites are gradually absorbed; whereas similar grafts with living cells retain their calcified matrix in soft tissue sites.

Dead autogenous tissue grafts with large amounts of non-living intercellular substance (cartilage, bone, fascia, and tendon) tend to be absorbed more slowly than dead autogenous grafts composed largely of cellular elements (epidermis, fat, muscle).

The gelatinous intercellular substance of cartilage is the most durable of all intercellular substances with the possible exception of elastin. Following transplantation, cartilage matrix may even persist for long periods of time as an autogenous graft when the cells in the graft have been previously killed by heat or other agencies. Dr. Gordon New (1) made practical use of this physiological fact when he advocated the use of "boiled autogenous cartilage grafts," which have less tendency to bend or become distorted. There is, however, a slow but progressive replacement of these dead grafts by host fibrous tissue, often associated with new bone formation. The author buried living segments of autogenous cartilage and nasal bone, and also heat-killed segments of autogenous cartilage and nasal bone in human abdominal fat. The patient returned four years later for another operation, which provided an opportunity to remove the grafts. The heat-killed cartilage and bone had completely disappeared but the living septal cartilage and bone with living cartilage and bone cells were present in about the same form and bulk. (See chapters on Cartilage and Bone Grafts.)

[1] It is possible that the agency (heat, alcohol and so forth) used to kill the fascia and tendon cells may alter the graft chemically and thus cause the graft to be rejected by the host tissues. It would be of value to kill the cells in autogenous tendon and fascia grafts by desiccation, immerse them in normal saline and then transplant the tissues.

IDENTIFICATION OF TYPES OF PARENCHYMAL CELLS

Cartilage

There is little difficulty in selecting the parenchymal cell of cartilage, for the tissue contains only one cell, which is surrounded by its own intercellular matrix. When cartilage is transplanted as a free autogenous graft, the surgeon wants the graft to retain its bulk, so that in saddle nose, in forehead depressions, and in total ear reconstruction, the nice contour established at the time of operation will also be present ten or even twenty years later. He may not appreciate the significance of the small living cartilage cells which he cannot see, but the ultimate success of his operation usually depends entirely upon the survival of the chondrocytes, which are the only living things in the graft. If the cells in a cartilage graft survive transplantation, they will usually continue to service and maintain their intercellular matrix, so that the bulk of the graft and the specific structure of the intercellular substance will tend to remain the same. If the cells fail to survive or, unhappily, are killed by heat before transplantation, the bulk of the graft tends to be slowly reduced and replaced by fibrous tissue or its derivatives.

Whenever a "definite statement" is made about the behavior of a free graft in this volume, the reader must recognize that occasional variations in behavior do occur. The statements made regarding the fate or behavior of free grafts after transplantation are based on what occurs in the large majority of grafts. Free grafts may change like established tissues, due to the physiological turnover which takes place in all living structures throughout adult life, and to other factors which are not known. Nothing living is actually stable for long periods of time.

Tendon and Fascia

The parenchymal cells in tendon and fascia are also easy to identify because these

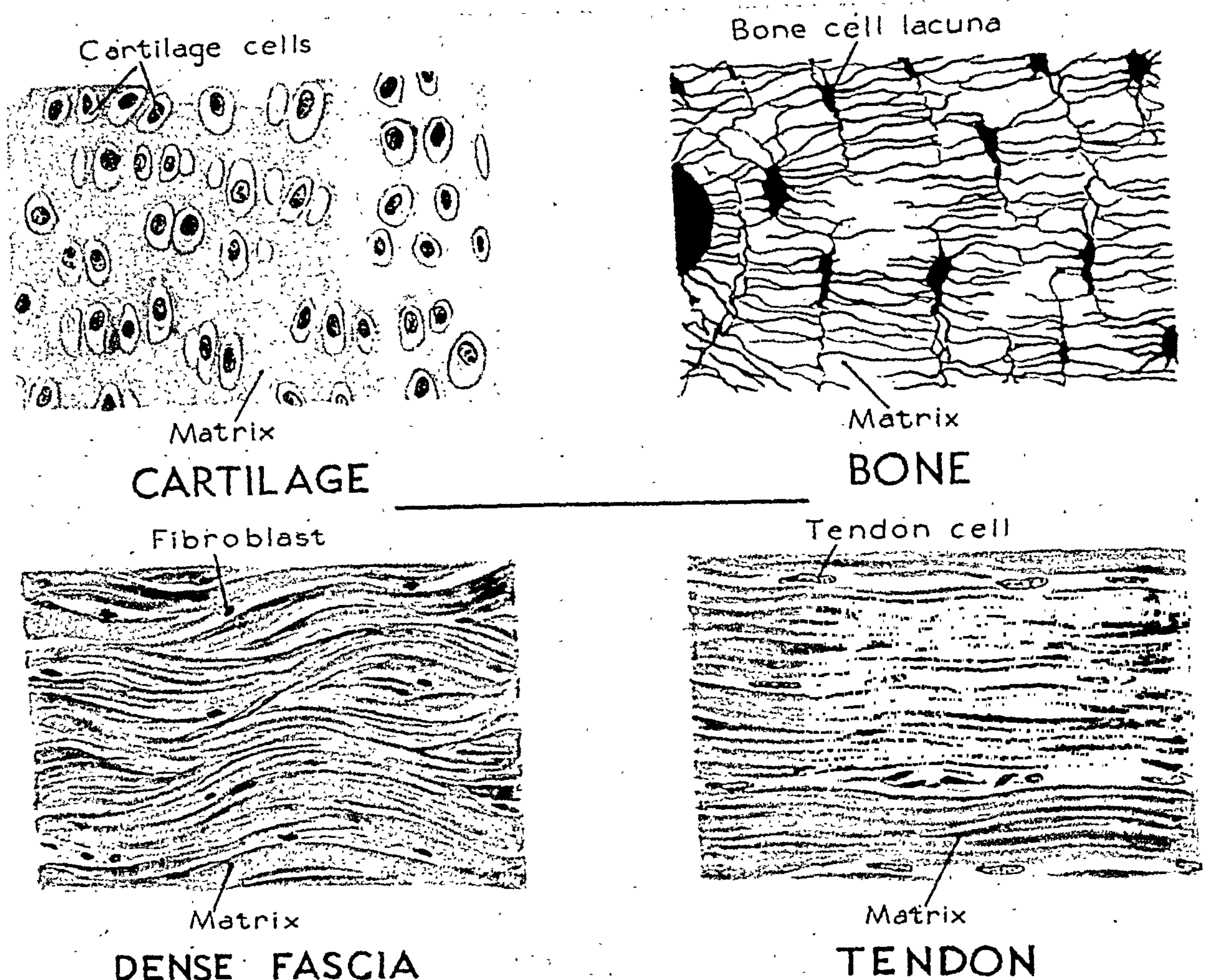


FIG. 15. Cartilage, bone, dense fascia and tendon. These tissues have similar basic structures in that they are composed of only one type of parenchymal cell, which is surrounded by a large amount of non-living intercellular material or matrix. This inanimate intercellular substance, which is the product of activity of the cells, determines the specific structure of these tissues. Thus, cartilage is firm but relatively elastic due to a gelatinous intercellular substance, bone is rigid due to a calcified material between its cells, and fascia and tendon are pliable and strong owing to the presence of tough bundles of fibers between the cells.

All of these tissues are derived from mesoderm and hence their respective parenchymal cells are descendants of primitive mesenchymal cells which have become specialized cartilage cells, bone cells, fascia cells or tendon cells.

All excepting cartilage contain blood vessels and nerves.

tissues resemble cartilage in having a single essential cell type supported and surrounded by intercellular material. This intercellular substance in tendon and fascia is composed largely of collagenous fibers. The intercellular substance of fascia appears to be retained when the fibroblast cell, which represents the essential or parenchymal cell, survives. The collagenous bundles in tendon also appear to be retained when the parenchymal tendon cells and stromal fibroblast cells[2] survive transplantation. No one knows what happens to the graft structure if the parenchymal tendon cells die and the stroma fibroblast cells survive or vice versa.

[2] Tendon bundles like nerve bundles are supported by a connective-tissue stroma with its own fibroblast cell, which is differentiated from the specialized tenoblasts or tendon cells.

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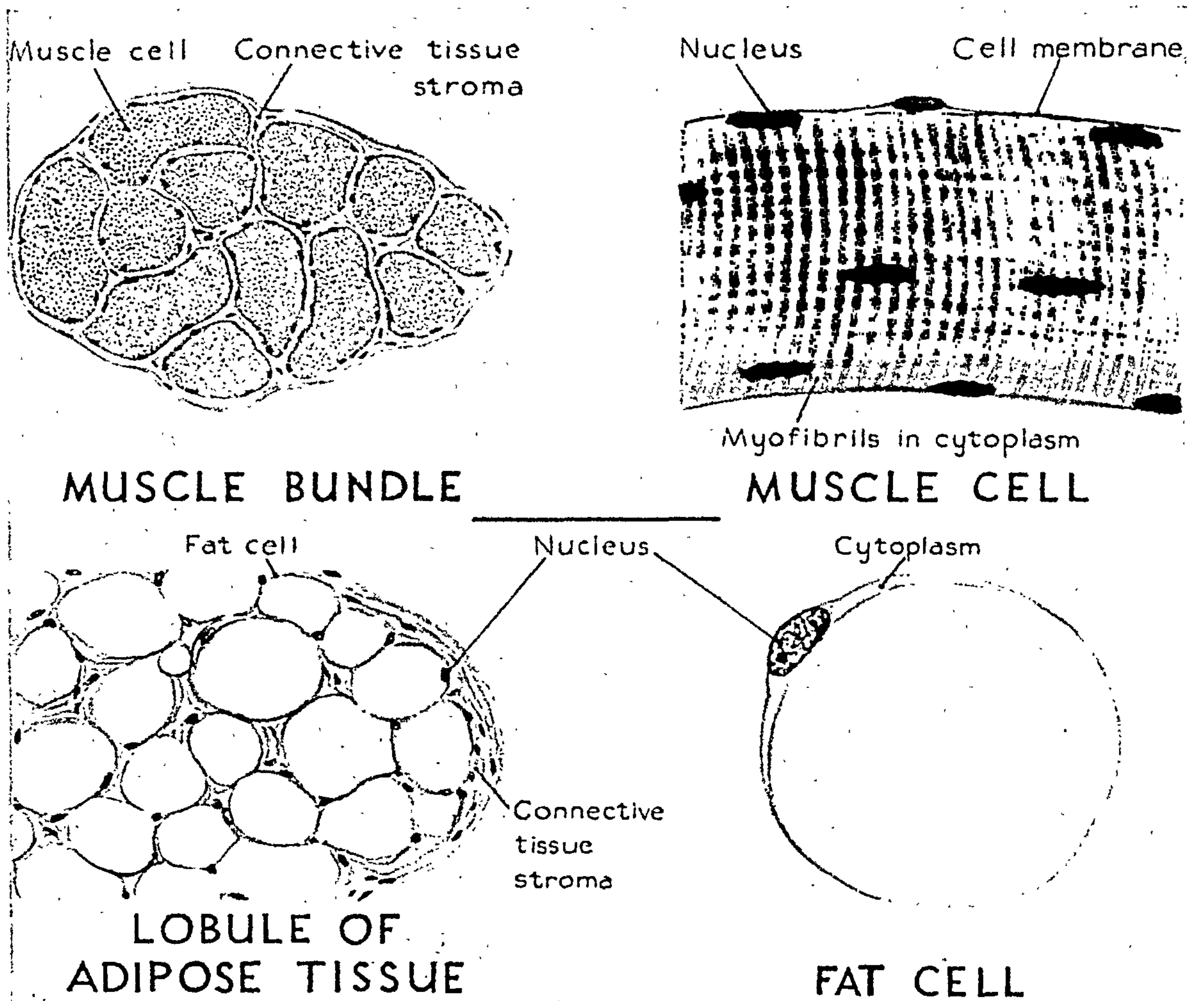


Fig. 16. Muscle and fatty tissue. These tissues are composed largely of cellular elements. They contain little inanimate intercellular material which is maintained by the activity of the muscle cells and fat cells. Thus the specific structure of muscle tissue is due to the muscle cells or fibers, which contain myofibrils and sarcoplasm, and the specific structure of fatty tissue is due to the fat cell with its content of fat.

Muscle and fatty tissue have a connective-tissue framework which serves as a supporting structure for the muscle and fat cells. The fibroblasts in this connective-tissue framework may survive transplantation when the muscle and fat cells fail to survive.

The skeletal muscle cell or fibers are multinucleated, thus differing from the cells in other tissues, which have a single nucleus. Recent work has demonstrated that the fibers in skeletal muscle may extend from the origin to the insertion of the muscle. Free muscle grafts therefore may be similar to the axon elements of free nerve grafts in that they consist largely of segments of cells rather than of complete cell entities. The axons and the muscle cells always degenerate and disappear in free nerve and muscle grafts.

Muscle and fatty tissue are derived from the mesoderm, and they contain blood vessels and nerves.

fail to survive, the graft, being on the body surface, sloughs away.

The dermis of the skin, however, may give us some difficulty. The sweat glands are very important physiologically, and the sebaceous glands and hairs are highly specialized structures. The connective-tissue dermis with its fibroblast cell appears to serve as a support for the glands and hairs and as a foundation for the epidermis. The dermis also contains specialized end-organs of sensation and pigment-forming cells, the

When the tendon and fascia cells fail to survive, the fate of the collagenous fibers is not definitely known. If the graft is in contact with unlike tissues (not tendon nor fascia) the fibers may disappear. When it is in contact with like tissue it is possible for the tendon and fascia to be replaced by creeping substitution as in dead bone grafts in contact with living bone. Future experimental work is required to clarify this point.

Bone

The bone cell is generally acknowledged to be the parenchymal cell in bone, with acceptance of the osteoblasts (or some other agency) as the builders of this tissue. The bone cell has the ability to retain its calcified matrix in certain types of bone grafts, with a complete absence of osteoblasts and osteoclasts, provided the osteocytes or parenchymal bone cells are viable.

We must roughly divide bone grafts into two types: those which have very little regenerative power (usually membranous bones) and other types which have regenerative power. Grafts of bone without regenerative power include nasal septal bone, the nasal bones, and the turbinates; these tend to retain their calcified matrix regardless of whether they are transplanted in contact with bone or with soft tissue, provided the parenchymal cells survive. When the parenchymal cells in these autogenous bone grafts are killed by heat before transplantation in soft tissues, the dead calcified intercellular matrix is slowly absorbed.

Other types of bone, such as rib, tibia, and iliac bone, which have regenerative power after injury, tend to lose their calcified structure when transplanted in soft tissues, but, alternately, tend to retain their structure when transplanted in contact with living bone. Such bone grafts in contact with bone become denser, so that they resemble compact cortical bone but they do not appear to increase in size or grow. The important function of the parenchymal cells in free autogenous grafts of septal, nasal and other facial bones is quite apparent. The role and relationship of the parenchymal cells in rib, tibia, and iliac bone are somewhat different in regard to survival of the calcified matrix; this will be considered later. (See Bone Grafts.)

Fat and Muscle

Fat and skeletal muscle have a connective-tissue stroma but the fat and muscle cells dominate the scene and serve the important functions of the tissues. The parenchymal cells, therefore, are the fat cell and muscle cell. The hardy fat cells which succeed in surviving after free autogenous transplantation tend to retain their small amount of amorphous intercellular substance. Muscle cells always degenerate and disappear after free transplantation, and the graft is replaced by fibrous tissue.

If the cells in a fat graft fail to survive, the graft will be replaced by fibrous tissue. Fat and muscle have a connective-tissue support of collagenous and elastic fibers, which extend between muscle cells and fat cells. This fibrous tissue has its own living fibroblast cells, and probably the dead amorphous intercellular substance surrounding the parenchymal cells also pervades and surrounds this fibrous tissue, since there is no recognized structure separating the two.

Skin and Appendages

The epidermis of the skin is similar to fat and muscle in that it contains very little intercellular material. Unlike fat and muscle it does not have a connective-tissue stroma supporting its cells and cell groups. The parenchymal cell is the epidermal cell and it tends to survive autogenous transplantation and retain its small amount of intercellular substance. When the epidermal cells

grafts to become so dark that they form a startling and unpleasant contrast with the surrounding skin.

Nerve

Identification of the parenchymal cell in a peripheral nerve graft also gives us some difficulty. The surgeon transplanting a free nerve graft is concerned with the growth of axons through the graft and on out to motor endings in muscle or to sense organs in the skin. These axons are cytoplasmic extensions or tails from nerve cells located in some remote ganglion or in the spinal cord. The severed nerve axons in a free nerve graft are destined to die in all instances because they have been disconnected from the cytoplasm of their nerve cells. Actually, when a free nerve graft is used to bridge a defect in some other nerve, the surgeon expects that new axons will grow through the graft from new recipient nerve cells. Consequently he removes the graft from an unimportant peripheral nerve which he is willing to sacrifice, and places it in contact with the severed axons of new

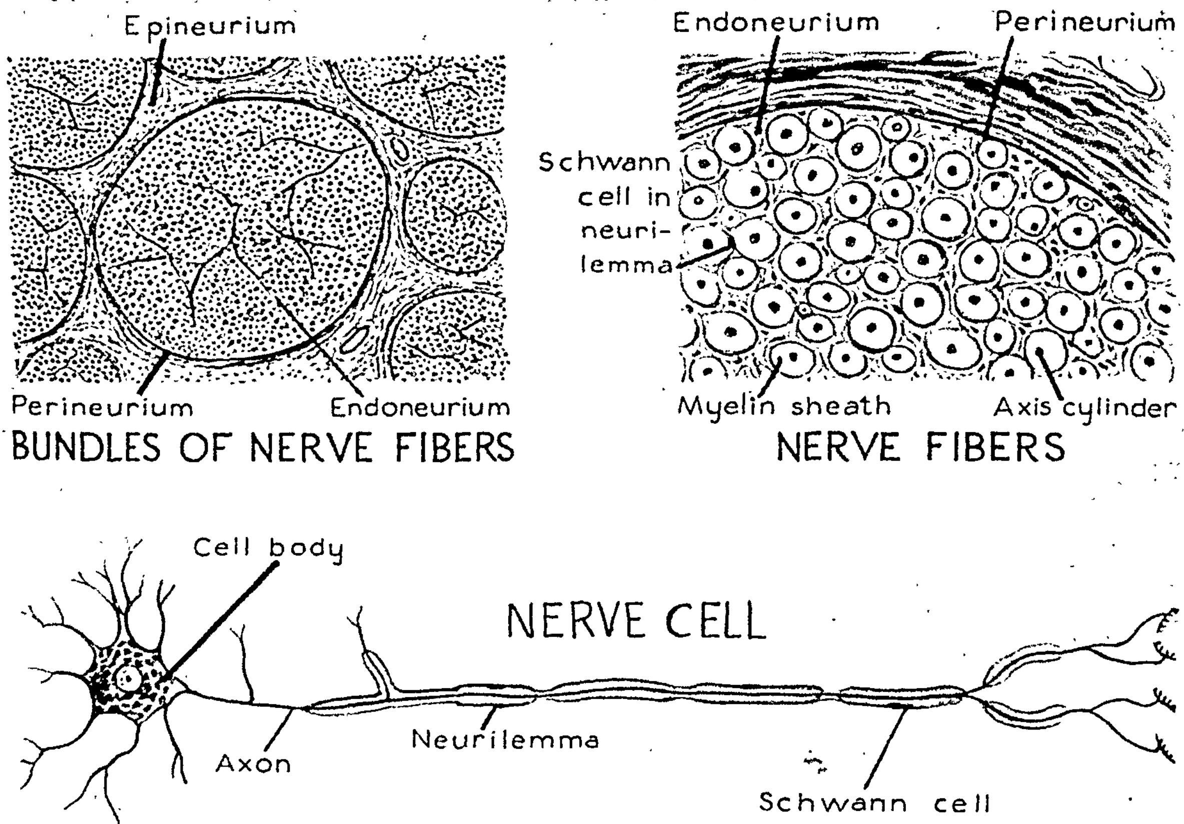


FIG. 18. Nerve. Nerves resemble skin because they contain cell and intercellular material of both ectodermal and mesodermal origin.

The parenchymal cell elements in nerve grafts, the axons (which are cell processes and not complete cells) and the Schwann cells in the neurolemma both are of ectodermal origin. The myelin sheath, which is a sort of non-living insulator for the axon, is believed to be produced by the Schwann cell rather than the nerve axon.

The presence of the Schwann cell is necessary for the normal functioning of a nerve axon and Schwann cells are seen growing out with regenerating nerve axons.

Nerve grafts also contain a connective-tissue framework of mesodermal origin, which is designated according to its location as epineurium, perineurium or endoneurium. A fibroblast cell is the parenchymal element in the neurium, and this cell may survive transplantation when the other elements in the nerve graft fail to survive, or disappear. Proliferation of these fibroblasts in the endoneurium often blocks the pathways through which axons attempt to grow.

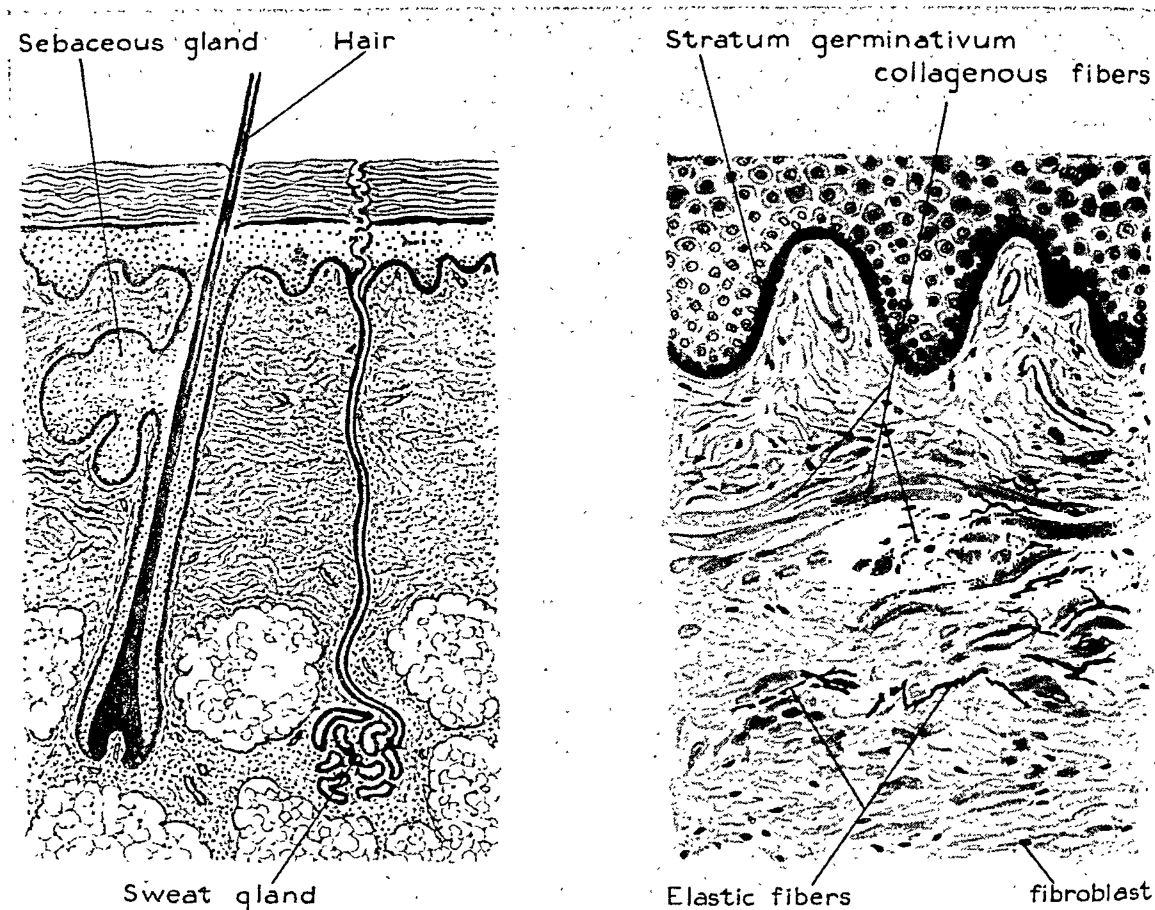


FIG. 17. Skin. This tissue consists of two different layers called epidermis and dermis. The epidermis resembles muscle and fatty tissue in that it is composed largely of closely packed epidermal cells, which give the tissue its specific structure.

The dermis resembles fascia since it has a single type of parenchymal cell, the fibroblast, which is surrounded by a large amount of inanimate intercellular material in the form of collagenous fibers and yellow elastic fibers.

The hair follicles, sebaceous glands, and sweat glands are within the dermis, and each has a distinct and characteristic parenchymal cell.

Thus in skin there are five different parenchymal cell groups, which are of different origins. The hair follicles, sebaceous glands, sweat glands and epidermis are of ectodermal origin. The fibroblast cell in the dermis, with its intercellular collagenous and elastic fibers, is of mesodermal origin.

The dermis contains blood vessels, while the epidermis resembles cartilage in that it is avascular.

melanoblasts, which, by increasing and distributing pigment, protect deep cells from excessive exposure to the sun. Thus in the case of the dermis we must concede that hair follicles, glands, and specialized nerve endings all have parenchymal cells. The melanoblast, which can do something no other cell can do, is a highly specialized parenchymal cell, whose absence gives rise to albinism.

The fibroblast, with its collagenous and elastic fibers, represents the supporting connective-tissue stroma for the parenchymal structures enumerated above. In general, the glands, hairs, and sense organs tend to survive in free autogenous skin grafts when the parenchymal cells in the structures survive. The melanoblast also tends to survive and may take on increased melanin production, causing free skin

grafts to become so dark that they form a startling and unpleasant contrast with the surrounding skin.

Nerve

Identification of the parenchymal cell in a peripheral nerve graft also gives us some difficulty. The surgeon transplanting a free nerve graft is concerned with the growth of axons through the graft and on out to motor endings in muscle or to sense organs in the skin. These axons are cytoplasmic extensions or tails from nerve cells located in some remote ganglion or in the spinal cord. The severed nerve axons in a free nerve graft are destined to die in all instances because they have been disconnected from the cytoplasm of their nerve cells. Actually, when a free nerve graft is used to bridge a defect in some other nerve, the surgeon expects that new axons will grow through the graft from new recipient nerve cells. Consequently he removes the graft from an unimportant peripheral nerve which he is willing to sacrifice, and places it in contact with the severed axons of new

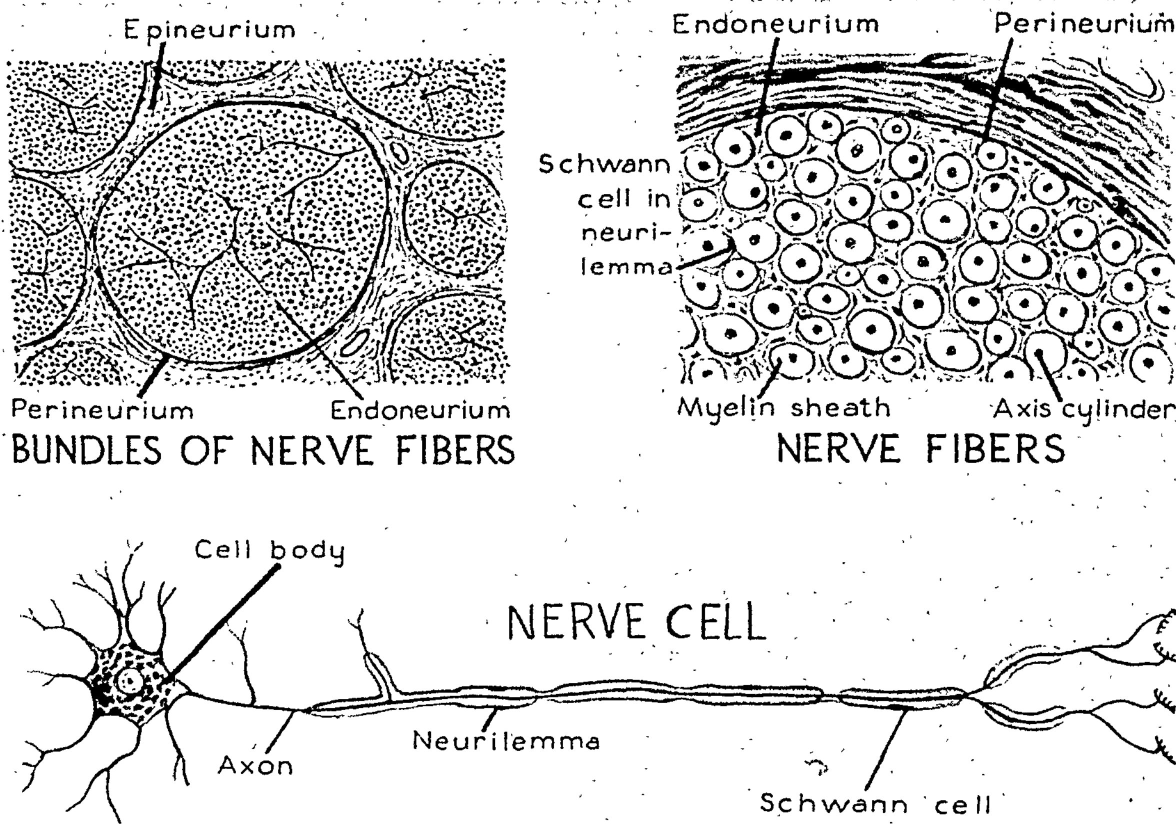


FIG. 18. Nerve. Nerves resemble skin because they contain cell and intercellular material of both ectodermal and mesodermal origin.

The parenchymal cell elements in nerve grafts, the axons (which are cell processes and not complete cells) and the Schwann cells in the neurolemma both are of ectodermal origin. The myelin sheath, which is a sort of non-living insulator for the axon, is believed to be produced by the Schwann cell rather than the nerve axon.

The presence of the Schwann cell is necessary for the normal functioning of a nerve axon and Schwann cells are seen growing out with regenerating nerve axons.

Nerve grafts also contain a connective-tissue framework of mesodermal origin, which is designated according to its location as epineurium, perineurium or endoneurium. A fibroblast cell is the parenchymal element in the neurium, and this cell may survive transplantation when the other elements in the nerve graft fail to survive, or disappear. Proliferation of these fibroblasts in the endoneurium often blocks the pathways through which axons attempt to grow.

important but remote nerve cell entities. This is robbing Peter to pay Paul, but a gain may occur in the exchange. From the clinical viewpoint the true parenchymal cell is the new foster parent nerve cell which may adopt the graft and provide it with new axons in the form of protoplasmic extensions from its cell body.

Being necessary for the normal functioning of a nerve axon, the Schwann cells in the neurilemma must be considered as parenchymal cells in peripheral nerve grafts. The myelin sheath, which is a sort of non-living insulator for the axon, is believed to be produced by the Schwann cell. The individual nerve axon with its insulating myelin sheath enveloped by the Schwann cell is supported by a connective-tissue stroma. This stroma, which is composed of collagenous and elastic fibers, has its own living fibroblast cell and is designated according to its location as endoneurium, perineurium or epineurium. Proliferation of the fibroblasts in the endoneurium of nerve grafts often blocks the pathways through which new axons attempt to grow.

In summary, we note that the individual nerve fibers with their myelin sheaths and enveloping Schwann cells resemble muscle and fat cells in that they are supported individually and in groups by a fibrous tissue framework containing its own fibroblast cells. Amorphous intercellular material surrounds the nerve fibers and also the supporting framework, and both the amorphous and fibrous intercellular materials permit exchange of necessary substances from capillaries to cells and vice versa.

The Schwann cell may be considered the parenchymal cell of free nerve grafts. The severed axons in the graft are segments of cells rather than complete cell entities and are destined to die. The myelin sheath is a non-living insulator, and the fibrous tissue stroma with its living fibroblasts serves as a support for nerve bundles. The success of a nerve graft depends upon axon replacement from a new and remote nerve cell, and on the survival or replacement of the parenchymal Schwann cell. The fibroblasts in the neurium always seem to survive free transplantation and proliferate to such an extent that they often block the pathways through which new axons attempt to grow.

In free nerve autografts transplanted in human abdominal fat the fibroblasts survive and proliferate to such an extent that the Schwann cells cannot be identified. In early sections the Schwann cells were clearly seen; they appeared as living cells. In later sections they could not be identified in the great multitude of proliferating fibroblasts. The axons and myelin sheaths degenerated and disappeared in all sections. Even eight months after transplantation the fibroblasts kept their fibrous tissue arrangement as endoneurium, perineurium, and epineurium. One cannot say that the Schwann cells died; they simply disappeared from view among the large numbers of proliferating endoneurial fibroblast cells.

REFERENCE

1. New, Gordon B., and Erich, J. B.: Method to prevent fresh costal cartilage from warping. Am. J. Surg., 54: 435, 1941.

CHAPTER 8

Heterogenous, Homogenous and Autogenous Grafts

PLANT GRAFTING

The attempts at vegetable grafting, which were the first to be made, go back a number of centuries. During these years grafting or budding has been employed for the propagation and perpetuation of tree fruits and also for the purpose of producing some radical change in the plant.

In plants not only is homografting successful but heterografting is a well-known procedure and consists in the union of the scion (graft) with the stock (root system or host tissue) of another plant when the difference in relationship is not too great. It must be emphasized that even though the stock and scion are in intimate union, each retains its own individuality. The bark, cambium layers, and other wood tissues of the two never mix but merely knit together.

The problems concerning just which plants may be grafted upon one another is still far from complete solution. Certain species graft with perfect ease; certain other species in the genus are united with difficulty, and apparent similarities are confusing. For instances, peaches do not bud readily on the apricot, but both the peach and the apricot may be grafted on the plum. The horse chestnut cannot be budded on the oak but the edible chestnut may be so united.[1]

Hottes (1) notes that absurd statements concerning graftage have been made continually by persons who have allowed their imaginations to rule their writings. Even Virgil speaks of apples growing on plum trees—a core fruit on a stone fruit. Botanists believe such things impossible. Martial speaks of grafting the cherry on the poplar. Madame de Genlis claims to have grafted the rose on the black currant to obtain "black roses." A prominent New York newspaper published, with apparent sincerity, an account of a "table d'hote" tree which, by grafting, grew tomatoes, cucumbers, potatoes, apples, and a dozen other

[1] The author is currently attempting to graft scions from blight-resistant Chinese chestnut trees with the stock of various oaks which are indigenous to the rocky soil pockets in Smoke Rise, New Jersey. In this locality the roots of the native American chestnut still send up suckers, which may grow as tall as 25 feet before they are affected by the chestnut blight and die. The durable American chestnut root system, which seems to be inexhaustible, promptly sends up new shoots to replace those which have been destroyed. It is possible that the American chestnut will gradually develop an immunity or resistance to the blight, as did the Chinese chestnut.

important but remote nerve cell entities. This is robbing Peter to pay Paul, but a gain may occur in the exchange. From the clinical viewpoint the true parenchymal cell is the new foster parent nerve cell which may adopt the graft and provide it with new axons in the form of protoplasmic extensions from its cell body.

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disintegration of homotransplanted skin, P. B. Medawar made many important observations and established a sound experimental approach to this subject. Further work is necessary, as the homotransplant problem is "not an obscure section of pure research"; there is, in addition, an increasing clinical demand for its solution.

That homotransplanted tissue disintegrates is not generally appreciated, excepting by a few experienced surgeons who have seen this happen clinically and have been induced to transplant various tissues in humans and study the behavior of their tissue transplants in fixed and stained sections.

Medawar, unfortunately, published most of his work in non-clinical journals during World War II, and these journals are not usually read by clinicians. Medawar has this to say: "The plastic surgeon has in the past relied on the laboratory worker neither for inspiration, principle, nor technical aid. I wish, therefore, to take the opportunity to review some of the significant work that has been carried out on homotransplantation in general and on skin in particular."

This statement regarding the lack of interest among plastic surgeons in the behavior of tissues applies also to other surgical specialists, and all appear to be entranced by methods of approach through complicated techniques rather than by simplification of these techniques to their basic essentials in harmony with the requirements and behavior of tissue cells. On the other hand, pure scientists can benefit by a closer relationship with surgeons, since this may suggest ways for clinical application of animal experimental work directed toward human needs.

Homotransplantation of tissue is successful in the lower orders of animal life but becomes increasingly unsuccessful as one ascends the phylogenetic scale. Resistance to foreign tissue *is most highly developed in the mammalian group.*

From the ontogenetic point of view, the embryo at certain stages has little or no resistance to alien tissue. This relative lack of resistance to alien tissue on the part of the embryo and the mild reaction to homotransplanted embryonic tissue have led to many exaggerated statements and great expectations. The method of approach, however, has disclosed many varied and interesting facts which are gradually being accurately evaluated.

HETEROGENOUS HUMAN GRAFTS

About the beginning of the century, as the use of skin grafts became popular, surgeons here and abroad developed an interest in the transplantation of other tissues for clinical purposes. Attempts at such transplantation were made for an amazing variety of conditions, with equally amazing results.

Little distinction was drawn between a graft of the patient's own tissues and grafts taken from other humans or even from animals. All apparently were good "takes" in the enthusiasm of the day. Gradually the more conservative surgeons gave up attempts to graft animal tissues in humans in most instances,[2] but homogenous transplants from one human to another remained popular.

In the light of our present knowledge it can be stated that animal grafts are not

[2] Ox cartilage and whale cartilage grafts are still in use. The author has buried cartilage from the giant sting ray in human tissues experimentally. Heterogenous arterial grafts have been used not only experimentally in animals but also for clinical purposes in humans when autogenous and homogenous materials were not available.

Preserved heterogenous bone grafts are implanted by some, and elephant ivory is occasionaly employed to support saddle nose and as pegs to fix bone grafts. Ox tendon and fascia are also sometimes advocated as grafting materials in humans but the general trend is to use autogenous grafts as the material of choice.

crops on one specimen. It was advised for planting in the small back yard. Thus, even with plants the successful union of their tissues is not entirely independent of systemic relation of the graft and host, and when the relationship is too distant there is protoplasm incompatibility and grafting fails. The degree of differentiation and specialization of the plant tissues concerned do not influence the results.

With plant tissue grafts as with animal tissue grafts it is necessary to prevent the cells from drying out. In plant grafts this is accomplished by sealing the cambium layer union of graft and stock by means of grafting wax.

The ability to grow and multiply is characteristic of all life and the object of the life of every plant and animal is to perpetuate its kind. Darwin realized this struggle for existence, and concluded that every fragrance, color, spine, tuber, and adaptation contribute toward the natural ability of the plant to live.

The seeds of plants, which are called ripe ovules, are actually embryo plants (2). This embryo developed inside the embryo-sac of the ovule from a fertilized egg-cell, just as if it were an embryo animal. Thus a seed turns out to be a very young plant, usually well-equipped with reserve food materials (on which we so largely depend) and surrounded by strong protective envelopes. As the developing seed remains for some time in close union with the parent plant, from which it gets its food materials, it may be compared without fancifulness to an embyro mammal developing inside its mother's uterus. In short, mammals and flowering plants have achieved *viviparity;* that is to say, what is liberated from the parent is a young creature already more or less advanced in development.

ANIMAL GRAFTS

The wide range of grafting successfully performed with plants cannot be accomplished with animals. In simple animal organisms (3)—for example, the hydra—heterografts can be made and animal chimera formed in which graft and host retain the characteristics of their own species, but in more complex organisms not only are heterografts impossible but homografts, with a few exceptions, also fail to survive.

Apparently as tissues become more specialized in the higher organisms these tissues lose their adaptability and cross-grafting becomes more difficult. That this specificity develops with the growth of the organism is demonstrated by the fact that embryonic tissues may be successfully cross-grafted but as development proceeds these embryonic tissues become more selective and cannot usually be transplanted from one animal to another.

John Hunter (4) was very interested in the behavior of transplanted tissues and carried out numerous experiments himself, though none of his personal observations on the higher animals are still valid for us today. His work on the homotransplantation of teeth, for example, was not confirmed by his contemporaries and fell into disrepute. However, although John Hunter has nothing illuminating to say on the homotransplantation of tissues, his experimental approach to the surgical problems remains an inspiration to modern surgery.

There can be no doubt that the problem of homotransplantation can be solved only by animal experimentation—a method fundamental in the teachings of John Hunter.

Dempster, a fine surgeon, is also unique for his broad perception of the cellular basis of life and its application to clinical problems in humans. His viewpoints regarding developments in homotransplantation are partly utilized in this chapter.

During the last war a stage was reached when there developed a great demand for skin replacement in burn casualties; there was therefore an urgent demand for a skin bank. Setting out to elucidate the constant

With few exceptions homotransplantation of skin has not resulted in permanent survival, and under ordinary circumstances the procedure has little place in clinical surgery at the present time. The few rare and authentic exceptions in which skin homografts were successful have amounted to about three instances (10–12).

Behavior of Homografts

A great deal of investigative work regarding the behavior of homografts is being done, and it is possible that in the future the problem will be solved. Some of the more important avenues of approach have been described by Longmire and Smith (13), and these various types of investigations are briefly presented with some additions.

A. Parabiotic Studies

It is possible to unite the bodies of two animals permanently by surgical means if they are members of a highly inbred strain (similar to identical twins). Attempts to transfer pedicled skin flaps between two humans with compatible blood groups have failed. Theoretically this would be possible in identical human twins. This is borne out by animal inbreeding studies.

B. Compatibility Studies

There are many blood types and probably many more which are not recognized; donor-recipient relationships, therefore, are not entirely controlled at the present time. Investigators have attempted to graft skin from donors who have blood groups compatible with recipients' blood by all of the known tests. So far this approach has not been successful in obtaining permanent takes of homografts.

C. Studies on Skin Groups

Although skin transfer does not react according to the principles of blood groups, there might possibly be other or similar additional factors governing compatibility, and different skin groups might exist just as do blood groups. Medawar (14) has investigated this possibility in rabbits and concluded that there are at least seven independently combined antigens governing the grafting reaction of rabbit skin. He suggested that a rabbit may belong to one of at least 127 skin transplantation groups. Longmire *et al.* (15) found evidence that there may be no less than twenty-three groups in man.

D. Experiments with Infant Tissues

It has been demonstrated that tissues can be interchanged between individuals of species low in the phylogenetic scale and between embryos of higher species. Reports have appeared in the literature indicating the permanent success of infant's skin transplanted as a homograft. The work of later investigators, including Longmire, has failed to substantiate these successful reports.

E. Studies on Altered Tissues

(*1*) *Effect of Freezing.* Tissues have been altered by freezing and storing at various temperatures from 45 to −108°F. for different periods of time and then transplanted as homografts [Baxter and Entin (16)]. The survival time of these homografts varied, but none survived beyond two to three weeks following transfer. These experiments indicate that reduced temperatures do not produce physiochemical changes in the cells of the graft that are beneficial to survival.

(*2*) *Tissues Altered by Heating.* Rouse (17) believes that causing hyperemia of the donor site by application of heat or by mechanical means increases the chances of the take of the homograft. Kiskadden and McDowell (18) have heated guinea pig skin homografts before transplantation and noted that the grafts so treated survive for

satisfactory in human tissues. Homogenous human grafts are better substitutes when autogenous grafts may not be used. One should qualify this statement, however, and not exclude the possibility that future work may discover some way to make animal tissue cells capable of surviving in human tissues or to influence the dead graft structure to remain. Some bacterial cells do equally well in animals and in humans and these bacterial cells may be considered to be foreign heterografts.

It appears that the cells and matrix in animal heterogenous grafts are not tolerated by human tissues. These cells, it is true, are dead cells at the time of transplantation, because all reported heterogenous grafts with the exception of a few surface skin grafts have been treated with preservatives or subjected to heat or other agencies before the grafts were transplanted. The dead cells and matrix, however, seem to incite antagonism in the host tissues so that eventually the foreign graft is absorbed or extruded.

HOMOGENOUS HUMAN GRAFTS

Although in the last century both Ollier and Thiersch observed that autotransplants of skin were most often successful, homogenous grafts continued to be used. This is easy to understand because fresh homografts of skin do take initially but tend grossly to disintegrate over a period of two to three weeks. Regeneration of the host epidermis from islands of dermis buried under the granulations could be mistakenly identified as the surviving graft. Regeneration of the patient's epidermis from the wound margins tends to surround a disintegrating skin homograft and this also may confuse the observer.

The behavior of buried homografts is still more difficult to determine excepting by long term observation. A fat graft transplanted from one human to another retains its bulk for some time, and the patient may go away quite satisfied. Other buried homografts such as cartilage and bone tend to retain their bulk for even longer periods; consequently the investigator, by observation and palpation, might logically decide that his graft had remained unaffected or had been replaced in kind by the surrounding tissues. The conclusions drawn by our immediate predecessors, therefore, were not out of keeping with the facts at their disposal, and they had the same proclivity to rush into print with a single case report that we see in our journals today.

Blood Group Factor

As the importance of blood groupings and compatibilities in transfusions became recognized, many investigators, believing that the riddle has been solved, began to apply this principle to homotransplantation. Thus, a number of successful skin homografts between compatible donors and recipients were reported. Recent works, however, demonstrate that skin homografts do not survive permanently regardless of the compatibility of bloods typed for the M and N subgroups as well as for the usual groups. Instances of successful homotransplantation of skin have been reported in identical twins (5–9) and it is now generally accepted that skin can be transplanted in such cases and survive permanently. *It is assumed that other tissues can also be successfully transplanted in identical twins but this has not actually been reported in humans.*

We have successfully transplanted a full thickness graft from behind the ear in identical twin boys with cleft palate. A switch was made so that each twin was bearing skin from the other. Both skin grafts were injected with India ink for later identification. The grafts took initially like autografts and could still be identified when last seen eight years after transplantation. The grafts behind the left ear of each twin still retained the injected ink.

tive response to homogenous tissues is less intense at this site than elsewhere in the body (29).

Theory of Actively Acquired Immunity

Any theory of an actively-acquired immunity would be obliged to demonstrate that a second crop of homotransplants from the same donor to the same recipient would disintegrate at a faster rate than the first. Using the skin of rabbits, Medawar (30) demonstrated exactly this; a second crop of homotransplants from the same donor to the same recipient did disintegrate at a faster rate than those of the first crop.

Dempster (4) remarks that Loeb and his school, in spite of repeated experiments, had not been able to show this, although they tried to find evidence of the reaction. Their failure can be explained by the fact that they used different donors for the second crop of homotransplants (31).

Medawar (29) also demonstrated that if a rabbit is specifically immunized against the skin of a given donor and later receives a skin homotransplant into the anterior chamber of its eye, the homotransplant will be destroyed only if it is invaded by blood vessels. Thus Medawar concluded: "The fact that vascularized eye implants are destroyed, though they are not dependent on a blood supply for continued survival, shows that vascular stagnation is not causally connected with the breakdown of skin homografts." It has been proposed by Medawar and others that first crop skin homografts become well vascularized and only later undergo disintegration. According to Dempster (4), kidney homotransplants show signs of disintegration before the blood supply fails. He also notes that blood vessel grafts, either embryonic or adult, do not appear capable of evoking an antibody response.

It appears to the author that different host sites vary considerably in their ability to provoke antibody response. The skin elicits the strongest antagonism to homografts, whereas the orbit, brain, and anterior chamber of the eye are especially favorable transplantation sites.

Perhaps too little attention has been given to the importance of using the behavior of the autograft as a yardstick in evaluating the behavior of similar homografts in like transplantation sites. *It is possible and even probable that a better understanding of the behavior of autografts would enhance our understanding of the fate of similar homografts.*

Favour (32) of Peter Bent Brigham Hospital, Boston, presented a very concise summary concerning the role of active immunity in the rejection of homografts. According to Favour, the one cell which is common in various methods of tuberculin allergy transfer *is the lymphocyte.* When the lymphocyte appears in quantity in a tuberculous focus, tuberculin allergy begins. A parallel destructive process in homografts is also coincidental with the appearance of lymphocytes at the site of the transplant.

Homografts by themselves can survive with the help of the fluid media of the recipient and this they do over a long enough period of time for the investigator to recognize that technical difficulties in establishing them in a new environment have been overcome. When they are subjected to invasion by recipient lymphoid type cells, however, allergy is presumably passively transferred to the graft. Since the graft is the source of the sensitizing antigens, it must first sensitize the recipient. As a rule, this sensitization takes several days or longer and may be delayed further (in experimental animals) by use of steroids, or by roentgenographic treatment, just as tuberculin allergy can be suppressed by treatment with steroids or roentgenographic therapy. Numerous other factors also affect survival time. For example, if the graft is

sixty to seventy days and longer after transplantation. A temperature of 50 to 55°C. for a period of thirty to sixty seconds is critical for the damage of skin cells in experimental animals (19).

(3) *Tissue Culture Methods.* Attempts have been made to alter the tissues of the graft in such a way that they would no longer be antigenic to the host. This was done (20) by growing the tissues to be transplanted in tissue culture with the recipient's serum—the purpose being that the donor cells might become adapted to their subsequent environment. In this way successful transplants of thyroid and parathyroid tissue of long standing were obtained. This is a very important contribution. Dr. Stone's article (20) should be read by all who are interested in homogenous gland transplants.

(4) *Embryonic Transplants.* Various types of tumor cells have been grown in chick embryos. Pieces of human skin have survived on the membranes of the chick embryo for as long as fourteen days, as reported by Goodpasture, Douglas and Anderson (21). The authors of this interesting work suggest the possibility of retransplanting such tissues back to man but report no instances in which this was done.

Various recent reports have appeared describing the successful transplantation of human embryonic or fetal endocrine gland tissue to young and adult humans. These transfers, which have been clinically successful in many instances, will be discussed in Volume II of this book, under the appropriate headings.

(5) *Tumor Transplants.* Transplantation of tumor tissue from one adult human to another has failed. Longmire and Smith (13) attempted to transfer a small squamous-cell carcinoma of the skin from one person to another with a similar type of lesion. They reported an initial "take" of the graft but it did not persist. Eventually, regenerated epithelium from the margins of the defect grew under the graft and the area healed with normal scar tissue. Thus, this interesting tumor transplant behaved like a homogenous transplant of normal skin.

(6) *Altered Host Reaction.* Desensitization of the recipient or host by the injection of large doses of donor serum was found by Baltzner and Beck (22) to be effective in prolonging the survival time of homografts. Conversely, Rohde (23) and others succeeded in shortening the survival time of homogenous grafts by treating recipient animals with blood plasma, serum, skin extracts, and skin autolysates.

F. Irradiation

Loeb's theory (24) of the cellular destruction of homografts may in part explain the disintegration of these transplants. He believes that host lymphocytes present in the general cellular reaction help to destroy the homotransplants. Irradiation, however, used as a means to reduce the number of lymphocytes has been unsuccessful in prolonging the life of homografts.

Total irradiation of the recipient animal has been reported to have prolonged the life of skin homografts (25–27), but one is still reluctant to apply this pretransfer therapy to humans considering its known harmful and dangerous effect and the late end-results. Administration of ACTH or cortisone to the recipient has prolonged the life of skin and other tissue homografts in animals. In humans the therapy has not been effective.

According to Foster and Hanrahan (28) the administration of antihistamine drugs to the recipient of a homograft resulted in the prolongation of the life of the graft up to ninety days. It is also known that a large variety of tissues, both autogenous and homogenous, will survive when transplanted into the anterior chamber of the rabbit's eye, and it would appear that the destruc-

tive response to homogenous tissues is less intense at this site than elsewhere in the body (29).

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small and hence liberates less antigen, survival may also be prolonged. Or, if the graft is placed in a location where cellular inflammation is slow to occur, as in the eye or brain, and where antigens are likewise slow to leave the grafted area, survival may be extended. So long as there is an opportunity for the lymphoid cells of the recipient to invade the graft, there is the potentiality of rejection on the basis of a tuberculin-type reaction.

If a graft does not stimulate a local cellular response beyond a certain minimum, transplantation may be possible. On the other hand, if anything happens to promote a local cellular infiltration, then a complex chain of events is set in motion. In a similar way, anything connected with the process of transplantation, such as trauma, infection, or the presence of local foreign bodies, which stimulates inflammation *per se* at the time of transplantation and thus localizes both cells and plasma factors at the transplantation site, will favor a subsequent allergic rejection of the homograft.

Survival Time of Homografts

Taylor and Lehrfeld (33) note that increased interest in the problem relating to homograft survival has emphasized the need for an accurate and reliable method of determining the duration of graft persistence. The series of events associated with the destruction of the graft, sometimes referred to as the rejection reaction, occupies a period of time generally extending over several days or weeks. A problem confronting the experimental worker therefore has been to decide upon an end-point for the measurement of survival time.

In measuring survival time, the most obvious and clinically the most often used end-point (with surface skin grafts) has been the occurrence of sloughing of the rejected graft elements. While this event can be easily recognized it fails to meet the more important requirement of an ideal indicator because it is a gross observation and varies considerably with different recipients of skin grafts. Taylor and Lehrfeld observed that circulation developed in rat skin homografts by the third or fourth day. Between the seventh and ninth day evidence of the onset of the incompatibility reaction was a stasis of blood in the graft blood vessels. That is to say, blood flow over the whole graft ceased although grossly the graft still appeared pink and healthy.

Some very recent incomplete observations on the survival of the cells in frozen skin homografts are described by Taylor and Lehrfeld in collaboration with Converse and others (34). The cells of a tissue in rats and mice are destroyed by freezing, without being previously treated. When the tissue is treated with a solution of glycerol or ethylene glycol before being frozen, however, considerable survival occurs, as judged by growth of the cells in tissue culture and by the cytological appearance of the cells as well as by the growth of hair in the graft when transplanted back into the original donor animal. *These are very important observations*, and Converse and his associates are to be congratulated on being among the first to use the tissue culture method for determining the viability of the cells in "bank tissues" and the successful autografting of frozen skin back again in the same animal. These methods of investigation should be utilized for all types of free tissue grafts on human volunteers.

The gross appearance and histological sequence of events in the temporary survival of skin homografts in human volunteers were studied by Rogers (34). He confirmed Medawar's observations in rabbits that homotransplants from the same donor to the same recipient in a second crop did disintegrate at a faster rate than those of the first crop. A marked local and systemic

eosinophilia was observed in full thickness skin homografts.

AUTOGENOUS HUMAN GRAFTS

Free autogenous grafts are always preferable to homogenous tissues as grafting material. When autogenous grafts are used the host reaction is less intense, healing is more rapid, and in all of the commonly used grafts excepting nerve,[3] muscle, and dense cortical bone the cellular elements in the graft tend to survive transplantation as living cells. It seems probable that these transplanted cells continue to live after transfer for their normal life span, which is not known in most instances.

Successfully transplanted autogenous cells also continue to maintain or are associated with the specific structure of their respective tissues. Thus bone (under proper conditions of transfer) remains as bone; cartilage, tendon, and fascia retain their specific structures; and skin remains as skin with its specialized glands, hair follicles, and nerve endings. The surviving cells in a free fat graft remain as fat and even retain their identity with their donor site since free abdominal fat grafts increase in bulk when the patient takes on an increase in abdominal fat.

Autogenous grafts, in the main, continue to live after successful transplantation as accepted and integral parts of the body anatomy. Some, like bone, will do better when they serve a functional use, whereas others like cartilage are quite comfortable almost anywhere provided they are buried and not exposed on the surface.

Certainly the patient's own tissue serves to provide the best of grafting material. Homogenous tissue grafts are second choice and serve only as a substitute when it is not expedient to use the patient's own tissues.

It is important always to bear in mind that there is *not a single instance where an autograft is not superior to a homograft as grafting material.* When a homograft is used clinically, there should always be a definite reason why an autograft could not be utilized. *At the present time there are no exceptions to this statement.*

[3] See free nerve grafts, Chapter 30.

CLINICAL USE OF HOMOGRAFTS VERSUS AUTOGRAFTS

In many instances homogenous corneal transplants appear to be a fair substitute for an autogenous corneal transplant. It is fortunate that this is so since there is no advantage in removing the graft from the patient's good eye. The observation that the surface epidermal covering of the corneal graft tends to be replaced by an ingrowth from the host cornea is of interest. The cells in the deeper connective-tissue layers of the cornea tend to survive, and the tissue retains its clear structure and does not usually become opaque, according to many authorities. Others believe that all cellular elements in the graft are gradually replaced by infiltrating host cells.

Living homogenous cartilage grafts often retain their non-living matrix for long periods of time. The cells appear to survive as living chondrocytes as long as they have a protective armor of matrix separating them from host tissues and hostile host antibodies or other substances. The lens of the eye also has the ability to survive as a fresh homograft. It is significant that the cells in all of these homografts which tend to survive are surrounded by a matrix substance rich in nucleoprotein (35).

Dead preserved homogenous cartilage grafts tend to retain their matrix but there is often a slow but progressive invasion and absorption of the graft structure. Sometimes, however, the grafts undergo rather rapid absorption, particularly in avascular scar tissue beds, where autogenous grafts may survive. Different patients may tolerate homogenous cartilage grafts, both living

and dead, in different ways. In some the grafts are well tolerated, whereas in others the graft structure tends to be absorbed rather rapidly.

Reports on dry-frozen cartilage grafts are not reliable because they have not been buried long enough to decide their ultimate fate. Preserved and dry-frozen homogenous blood-vessel grafts are used with apparent clinical success by vascular surgeons.

In general, homogenous cartilage grafts are useful as a readily available grafting material when it is not expedient to use the patient's own cartilage. In young individuals with long life-expectancy it is wise to use autogenous cartilage rather than homogenous cartilage.

Refrigerated and preserved homogenous bone grafts also have a field of usefulness. These dead grafts in contact with living bone serve as a scaffold for the formation of new bone by creeping substitution from the living host bone. Orthopedic surgeons use homogenous grafts frequently but the majority of plastic surgeons favor living autogenous bone grafts.

SUMMARY

Preserved heterogenous grafts have little place in clinical surgery at this time. The graft structure is slowly absorbed as in ox cartilage and giant-ray cartilage, or it will be rapidly extruded or absorbed in the more cellular preserved heterogenous tissue grafts. Heterogenous bone grafts are used by some, and it is stated that they may be absorbed and replaced as bone by host tissues. They are inferior to homogenous bone grafts, however, and sometimes the former are extruded.

There are no reliable reports in the literature on the transplantation of fresh heterogenous grafts with living animal cells in humans.

In general, one can state that no investigator has demonstrated that the cells in living homogenous transplants survive for long periods of time excepting possibly cartilage cells, the cell in the connective-tissue part of the cornea, the lens, and certain red blood cells in transfusions.

Preserved homogenous cartilage grafts are useful because the intercellular matrix often serves as a well-tolerated foreign body, even though the cells are dead. In homogenous bone grafts the calcified matrix serves as a framework which is invaded and replaced by cells from the living bone with which it is in contact or from other host tissues. Certainly no one has demonstrated that the cells in living homogenous bone grafts survive transplantation as living cells.

In peripheral nerve repair it would be advantageous to use homogenous nerve grafts. One could then select a graft of proper size to suture between the two ends of the living nerve instead of using a bundle of small autogenous nerves as a cable graft to bridge the defect. Most authorities at this time, however, prefer autogenous nerve grafts, living or dead. They believe that axons will grow down through poorly-fitting autogenous cable grafts better than they will grow down through a nicely-fitted single homogenous nerve graft. The field of peripheral nerve grafting has not been too well explored, and many changes may occur in the future, because the clinical results of free nerve grafting are frequently disappointing.

Since all available evidence demonstrates that skin homografts do not survive permanently, their clinical use is indicated only as a temporary covering for the severely-burned patient to tide him over his critical period.

Borst (36) in 1913 ascribed the failure of homogenous and heterogenous transplantation to the extreme specialization of the cell in the higher animals. Each species and, indeed, each individual must be regarded as

a specific biochemic system, so that with each advance in phylogenesis there emerged a specifically constructed and a specifically reacting system. On this basis Borst postulated a theory of "biochemic difference" to account for the opposing forces to homotransplantation and heterotransplantation. In homografts the maintenance of the graft itself depends on the existence of the least possible biochemic differences between recipient and donor. He further held that biochemic differences exist not only in members of different species but also in those of the same species, and even of the same race. One is justified in speaking of an "individual" as an "indivisible" one.

Leo Loeb later developed his tissue specificity theory, probably based in part on the pioneer work of Borst.

Medawar (29) postulates that homografts liberate some product, possibly from the nuclei of the cells in the graft. This substance in homogenous skin transplants stimulates the formation of a specific antibody, which is then carried through the blood stream back to the graft. Here it reacts with the nuclear substance during division of the cells, causing destruction of the cell and gradual necrosis of the graft.

Buried homografts such as bone and cartilage have few cells in contact with the host tissue fluid and hence give rise to little reaction. Buried cellular homografts such as fat and dermal grafts cause greater reaction and are completely absorbed in short periods of time.

Fresh gland homografts (without immediate surgical blood vessel anastomosis) appear to remain viable for about the same period of time as fresh skin homografts, which fits in nicely with Medawar's antibody theory. Probably other fresh soft tissue homografts in man behave in the same manner.

A very important observation made by Bacsich and Riddell (37) is as follows: Fresh homogenous grafts of cornea, lens, and cartilage resist absorption and the cells remain viable because these tissues are avascular and because their gel-like matrix is rich in nucleoprotein, which serves to protect the cells from hostile host antibodies. In other homogenous tissue grafts, such as fat and skin, and in organ transplants, which are vascular and not sufficiently protected by gel-like mucoprotein, the cells are destroyed in short periods of time, presumably by hostile host antibodies, and the grafts disintegrate.

Wyburn (3) notes that the capsule of the pneumococcus contains a mucoid substance similar to that in the intercellular substances of cornea, lens, and cartilage. The pneumococcus, which may be considered to be a foreign heterograft in human tissues, protects itself against hostile host antibodies by increasing the thickness of its mucoid capsule. It is suggested that the significant factor in the survival of homogenous tissue grafts is the presence or absence of mucopolysaccharides, particularly the hyaluronic-acid-hyaluronidase system. The resistance of skin homografts to host reaction for some time may be due to the presence of a hyaluronic-acid gel in the intercellular substance of the dermis.

One rather universal example of successful homografting which has not been mentioned *is the transplantation of the homograft male spermatozoa into the female ova*. This differs from ordinary tissue transplantation in that the host site is intracellular. The process of fertilization is somewhat similar to the action of a large virus (heterograft?) which becomes transplanted into the cytoplasm of a susceptible cell and remains viable.

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PART II

Cartilage

CHAPTER 9

Structure of Cartilage

Cartilage, a specialized dense connective tissue, is derived from mesenchyme, which is a thin mixture of cells arising from the embryonic mesoderm.

The undifferentiated mesenchymal cells are assigned various roles by an unknown agency, called "embryonic organizer," in the development of the human body. Some become specialized as cartilage cells surrounded by a gelatinous matrix, whereas others become fascia and tendon cells with tough bundles of collagenous fibers between the cells. Other similar-appearing mesenchymal cells, by means of changes in their cytoplasm, take on the characteristics of elongated muscle cells or swollen fat cells.

GENERAL CHARACTERISTICS

Cartilage is the dominant tissue in most of the embryonic skeleton, provides a framework in which most bones develop, and is an important factor in the growth of many bones. In the adult human it persists as cartilage, which provides a surface for bony joints, and a structural support for the ears, nose, respiratory tract, and anterior chest cavity, where it is present in the greatest quantity in the adult.

Cartilage resembles cornea and epidermis of the skin in that it usually contains no blood vessels. Vessels supplying other tissues occasionally pass through cartilage [Bloom (1)] and whenever bone formation occurs, blood vessels penetrate the cartilage to supply the bone.

By means of serial sections I have been able to trace the path of new blood vessel growth into fresh autogenous and homogenous cartilage grafts. These vessels probably serve as supply channels for invading host fibroblasts and other host cells, or for areas of new bone formation. The blood vessels do not seem to penetrate large cartilage autografts merely to effect better nourishment for the centrally located cartilage cells.

Following transfer, fresh cartilage grafts and epidermal grafts apparently retain their normal mechanism of nutrition by osmotic diffusion of tissue fluids from the host bed into the graft rather than the method of penetrating capillary ingrowths. In free transplanted skin grafts, blood vessels penetrate the dermis but do not enter the epidermis, which also retains its osmotic method of exchange.

TYPES OF CARTILAGE

Three chief varieties of cartilage are distinguished. *Hyaline cartilage* is characterized by a matrix or intercellular substance that is almost clear and free from obvious connective-tissue fibers. The other two types of cartilage are termed *fibrocartilage*

because they are characterized by a matrix pervaded by connective-tissue fibers. When these fibers are of a white variety, the tissue is called *white fibrocartilage.* When they are yellow elastic fibers, the tissue is called *elastic fibrocartilage.*

Hyaline cartilage is the type most commonly found in the human body and is the most typical, or true cartilage, the other types being modifications of it.

Hyaline Cartilage

Hyaline cartilage, the most prevalent form in the human embryo and adult, is present as rib cartilage, nasal septum, lateral and alar cartilage, and as the articulating surfaces between bones. The interarticular cartilages, often removed in corrective orthopedic operations, are fibrocartilaginous.

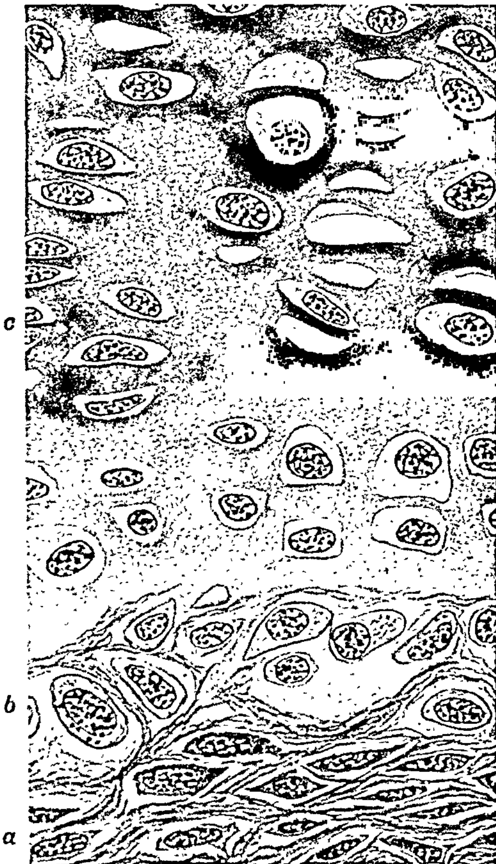


Fig. 19. Hyaline cartilage from the xiphoid process of a rat: *a*, Transition layer adjacent to perichondrium; *b*, continuation of collagenous fibers from the perichondrium into the interstitial substance of the cartilage; *c*, columns of isogenous groups of cartilage cells, some of which have fallen out of the cavities. Eosin-azure stain. 750X. (A.A.M.) From, A Textbook of Histology, 5th ed., Alexander A. Maximow and William Bloom. Philadelphia & London: W. B. Saunders Co., 1948.

Cartilage is unique among other tissues in containing only one living unit—the cartilage cell—which apparently is the parenchymal cell since it has no competitors. Cartilage is completely free from any of the wandering cells found in most other tissues and contains no blood vessel, lymphatic or nerve cell elements.

Cartilage Cell Types

The cells or chondrocytes occupy small spaces in the intercellular substance called lacunae, which are surrounded by relatively large areas of glassy homogeneous matrix. Centrally located cells are usually rather large and spherical in form. They occur singly or in groups of two, four, eight and so forth; these groups have originated from the division of a single cell, the first subdivision resulting in the production of two cells, and each of these again dividing into two cells and so on. Toward the peripheral surface, cartilage cells become spindle-shaped, and the outermost layer of cartilage contains cells that resemble rather plump fibroblasts.

Examination of Cartilage Cells

In *fixed sections* of fresh cartilage the more centrally located cells often seem to be retracted from their cell walls, but the peripheral spindle-shaped cells show little or no contraction.

Hyaline and other types of cartilage can best be studied in fresh *unfixed sections*, so that cartilage cells are seen in the living state and not as "cadaver cells." The physical properties of cartilage make it unique among the tissues in permitting sectioning and examination in the living state. It is quite easy to immerse fresh

cartilage in paraffin and cut sections of the living unfixed tissue with the microtome or slice it manually. These sections, immersed in normal saline, can be studied under the microscope and the cells observed in their living state. One can introduce supravital dyes under the cover slide and observe the accuracy of staining reactions of cartilage. As the saline evaporates, cartilage cells can be seen dying from desiccation and retracting from the walls of their lacunae, thus resembling dead cells in fixed sections of cartilage.

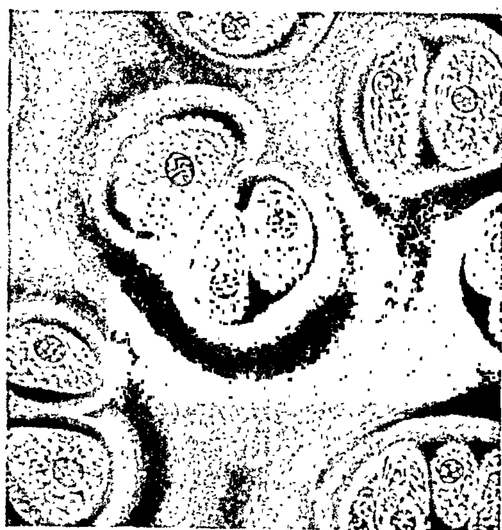
During life the cells appear to fill the tiny lacunae entirely but sometimes a small clear space can be distinguished between the cell and its matrix wall, so the cell seems to be floating in the intercellular fluid which fills the lacuna.

The *cytoplasm* of cartilage cells contains numerous fat droplets, long mitochondria and vacuoles, which give it a varied and interesting appearance. The *nucleus* is sharply differentiated from the cytoplasm and appears very faintly stippled and rather homogeneous in fresh unstained sections.

Stained sections demonstrate the same specialized structures found in other cells, e.g., one or several nucleoli in the nucleus, a Golgi apparatus etc. Mitotic figures are seldom seen in adult cartilage. Cell groups in adult cartilage are separated by relatively large areas of hyaline matrix, whereas embryonal and young cartilage contain less matrix in proportion to the number of cells.

Intercellular Matrix

The intercellular substance of hyaline cartilage appears homogeneous in fresh sections and in ordinarily fixed specimens, because the index of refraction of collagenous fibers and that of the binding material about them are the same. The matrix immediately around the lacunal chambers, however, takes a deeper stain and has concentric striations. These areas



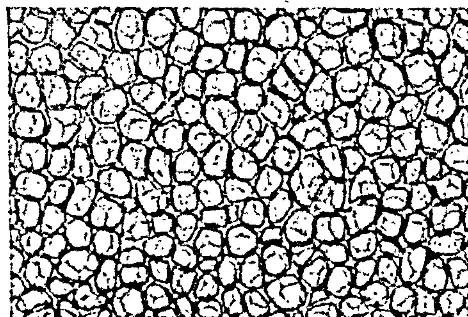




FIG. 20. A. Hyaline cartilage of a calf. (Redrawn and modified after R. Krause.) 400×.

B. Cellular cartilage containing very little ground substance, from the ear of a mouse. Dehydrated and mounted in damar. 100×. (After Schaffer.)

C. Portion of a tracheal cartilage of a guinea pig from which all constituents except the collagenous fibers have been removed by digestion with trypsin. (After Ruppricht.) From A Textbook of Histology, 5th ed., Alexander A. Maximow and William Bloom. Philadelphia & London: W. B. Saunders Co., 1948.

which appear to surround the cartilage cells are called cartilage capsules, and represent the youngest matrix elaborated by the cells.

When homogeneous cartilage matrix is treated by silver impregnation or by digestion with trypsin [Maximow and Bloom (2)] collagenous fibers may be seen; these fibers may interweave as a dense feltwork and run in all directions, or they may lie in definitely-oriented bundles.

One often detects collagenous fibers in hyaline cartilage grafts of various types. Apparently in such grafts a breakdown in the binding material has occurred which allows the collagenous fibers to be seen. Thus, the intercellular matrix of hyaline cartilage, which appears homogeneous, actually contains both formed and amorphous types of intercellular substance. The formed elements are represented by collagenous fibers, which are immersed in a relatively large quantity of amorphous intercellular substance with an identical refractive index. This amorphous intercellular substance is one of the sulfated mucopolysaccharides (described in the chapter on Intercellular Substances) and is known as chondroitin sulfuric acid. It is probably bound to a protein which is as yet unknown [Ham (3)].

Elastic Cartilage

This variety of cartilage is present in the external ear, the ear canal, the eustachian tube, the epiglottis, and in parts of the cuneiform corniculate cartilages.

Its cells are like those of hyaline cartilage but the intercellular substance contains numerous branching elastic fibers. These fibers can be clearly seen and they frequently form a dense network that obscures the amorphous substance in the matrix. Elastic fibers within the cartilage itself continue or extend into those of the perichondrium, which explains to the plastic surgeon why it is so difficult to remove the perichondrium from elastic cartilage of the ear.

Fibrocartilage

Fibrocartilage in the interarticular cartilages of the knee joint is frequently removed by orthopedic surgeons for "water on the knee." Recently Vidaurre (4), Mir y Mir (5), and Barkus (6) have used interarticular fibrocartilage as a free grafting material. Fibrocartilage also occurs in the intervertebral discs, in the symphysis pubis, and in the places of attachment of certain tendons to bones. Fibrocartilage cells resemble those of hyaline and elastic cartilage but the intercellular substance contains thick compact collagenous bundles lying parallel to one another like the collagenous bundles in tendon. The cartilage cells themselves are located in long narrow clefts, encapsulated between the thick collagenous bundles.

It should be noted that the tarsal plates of the eyelids are composed of dense connective tissue rather than fibrocartilage.

NUTRITION

Since cartilage contains no blood vessels or lymphatics, nutritive fluid from blood vessels in the perichondrium must pass through the solid-appearing matrix to reach the cartilage cells. The system of liquid-conducting canalicules described by early investigators is now believed to be an artifact. Because a non-toxic dye is rather quickly and evenly absorbed by this matrix, it seems apparent that fluids can pass through this intercellular material [Maximow and Bloom (2)]. The exact mechanism of the process is unknown, but there is no doubt that it does occur.

In synovial or joint cartilage the mechanism of nutrition is more complicated. Circulation may be accomplished by diffusion from underlying bone, or by diffusion from the synovial fluid, or a combination of both.

The mechanism of the diffusion is also not known.

When hyaline matrix becomes calcified, its normal nutrition by fluid permeation is interfered with and the cartilage cells frequently die. These calcified areas are often invaded by blood vessels and the dead calcified cartilage replaced by bone.

GROWTH OF CARTILAGE

Normal growth of all cartilage elements during childhood takes place in the deep layer of connective-tissue cells in the perichondrium which surrounds cartilage. After cell division some of these connective-tissue cells differentiate into chondroblasts which, surrounding themselves with hyaline intercellular material, become chondrocytes. The exact mechanism of this hyaline matrix formation is vague, but it is possible that the process is similar to the production of collagenous fibers by fibroblasts in healing wounds, as described by Stearns (7). This increase in the size of cartilage by means of cellular and matrix outgrowth from the deep connective-tissue layers of the perichondrium is known as appositional growth.

Growth also occurs by division of the deeper cartilage cells, followed by production of matrix about each cell, separating one cell from another. During adult life cartilage ceases to grow, and there is considerable doubt that it has any power to regenerate following injury. In the adult the cartilage wound is filled in with connective tissue associated with little, if any, new cartilage formation. It is well known that new cartilage formation does not occur following removal of costal cartilages, even though the perichondrium is kept intact and remains in place. This unfortunately is also true in the infant and young child as well as in the adult. The author has removed complete rib cartilages subperichondrially in three-year-old children for use in the reconstruction of both external ears, and on exposing the donor area eight and ten years later, has not noted any evidence of new rib cartilage having been replaced by the original intact perichondrial sheath.

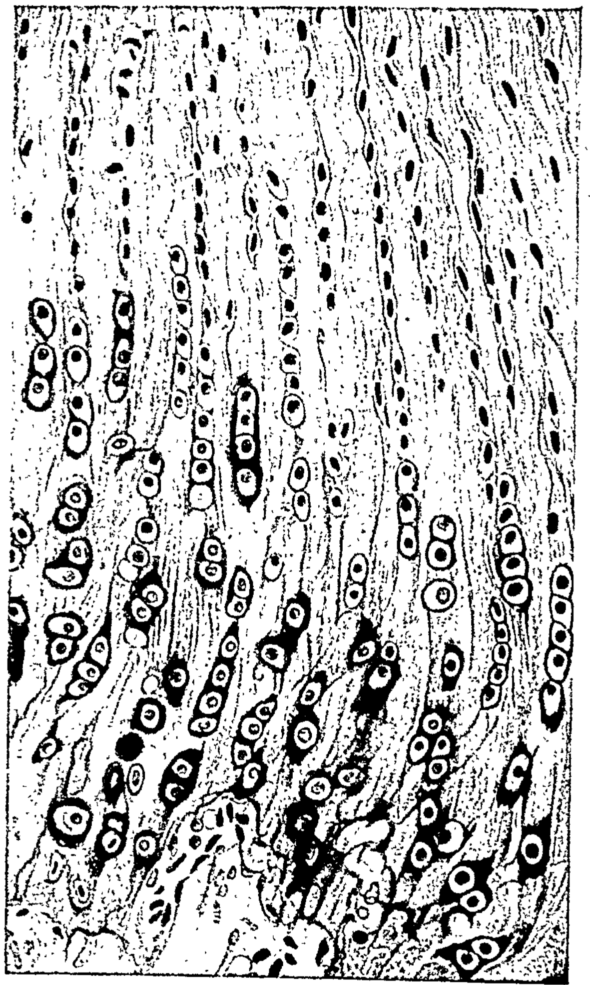


FIG. 21. Low power drawing of insertion of tendon into the tibia of a rat. Note the direct transformation of the rows of tendon cells (above) into cartilage cells surrounded by deeply staining cartilaginous matrix. Hematoxylin-eosin-azure II. From a preparation of F. C. McLean. (Drawn by Miss A. Nixon.) From A Textbook of Histology 5th ed., Alexander A. Maximow and William Bloom. Philadelphia & London: W. B. Saunders Co., 1948.

Septal cartilage removed subperichondrially in infants and young children is also not replaced, and this applies likewise to alar cartilage and to elastic cartilage of the ear. The failure of alar and lateral cartilages to be replaced following removal has influenced

many surgeons to avoid any removal of these cartilages during operative procedures in harelip repair. Some surgeons even avoid any undermining of the alar and septal cartilages in harelip procedures, because they believe that undermining may interfere with the normal growth processes of cartilages.

Certainly the septal cartilage removed subperichondrially in the submucous resection procedure in children and adults is not replaced by new cartilage formation. Thus it appears that the orderly growth process of cartilage structures which takes place with great regularity in the growing child can be easily disrupted by removal of portions of cartilage although the perichondrium is left in place.

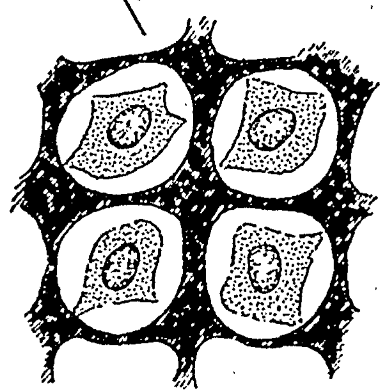
Dupertuis (8) reported appreciable growth in young autogenous rabbit cartilage grafts buried as free grafts with and without perichondrium. In early studies the author also thought that he had observed growth in several small autogenous human cartilage grafts. In later observations, grafts buried for longer periods of time, however, showed no evidence of growth. In a child, three and a half years of age, a complete but short segment of rib cartilage which had been completely detached from its perichondrial bed was then replaced in this bed, and the perichondrium was sutured over this rib cartilage. When the chest cage of this child was opened four years later to obtain additional cartilage, the rib segment was obviously present but it had been joined with the adjacent posterior rib cartilage by fibrous tissue rather than by cartilaginous union. In another young child the perichondrium had been thoroughly removed from a complete and intact rib cartilage but the continuity of this cartilage was not disrupted. It was not excised from the patient; as a result it appeared to have developed quite normally. Thus, on the basis of a single case experience the growth of cartilage seems to depend upon *continuity of the cartilage.* The perichondrium is not as active a structure therefore as rib periosteum, which may have the capacity to regenerate its specific tissue, bone, both in children and in adults.

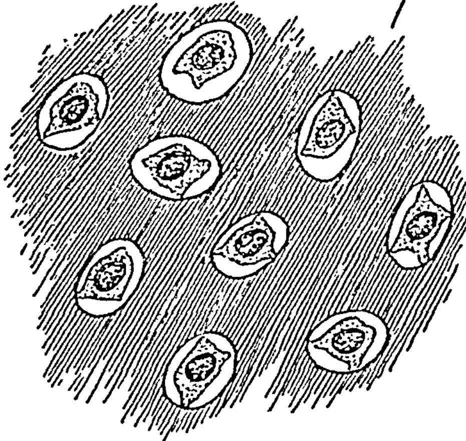
Certainly the perichondrium is important for appositional growth of undisturbed cartilage structures but it does not seem to have the ability to replace segments of cartilage that have been removed. Continuity of a cartilaginous structure seems to be a material factor for normal cartilage growth in the human. Moderately large segments of septal cartilage may be removed without interfering with the apparent normal growth of the nose in a child if the continuity of the septal cartilage support is maintained. When this continuity is disrupted or broken, the normal growth of the nose may be adversely affected. Bisgard (9) noted an absence of cartilage regeneration after the resection of complete rib cartilage in nineteen adult patients, although the perichondrium had been carefully left in place. In experiments on dogs he transplanted rib bone segments into the perichondrial sheaths and observed that the bones fused together to form a solid structure giving support to the chest wall. These findings suggest the clinical use of iliac bone segments seeded in the perichondrial sheaths of humans where large amounts of costal cartilage have been removed for reconstructive and other surgical procedures.

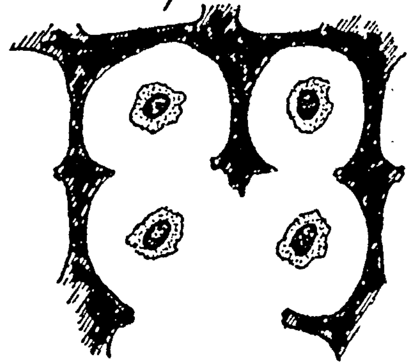
Cultivation of Cartilage in Vitro

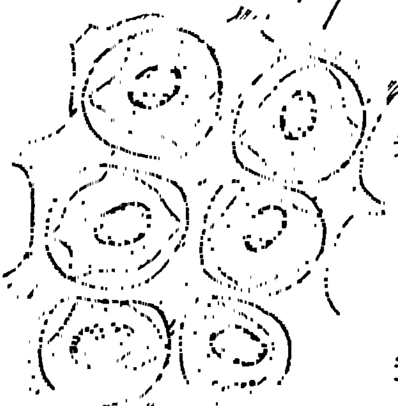
Cultivation of small fragments of cartilage usually results in the loss of chondroitin sulfate from the matrix and reversion of the cartilage cells to fibroblast-like cells. Cultivation of complete cartilage segments with their enveloping perichondrium permits the slow growth of cartilage containing chondroitin sulfate in its matrix (10, 11).











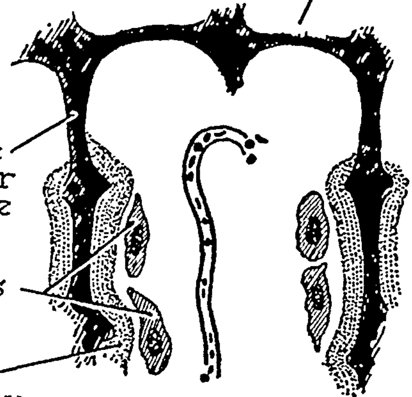


Fig. 22. Diagrams to show the development, life history and usual fate of cartilage in the body. From Histology, Arthur Worth Ham. Philadelphia: J. B. Lippincott Co., 1950.

Types of Cartilage Used as Transplants

Cartilage tissues of the hyaline variety commonly used as grafts include rib cartilage, and septal, alar and lateral cartilage of the nose. Elastic ear cartilage is occasionally employed as graft material, and recently the use of interarticular fibrocartilage has been reported. The behavior of hyaline, elastic and fibrocartilage after transplantation appears to be almost identical. This applies similarly to autogenous, homogenous and heterogenous grafts of the same types of cartilage.

Cartilage Matrix without Cells as Grafting Material

The possibility of removing the chondrocyte from cartilage and utilizing the matrix as a sort of "ideal homograft" material without antigen quality, is fascinating but illusive. Attempts to synthesize a cartilage matrix material have not been successful. Workers at the Peer Clinic are currently attempting to separate the cells from the matrix by a different approach and this may some day be accomplished. Homogenous and even heterogenous cartilage matrix without cells might provide a valuable grafting material, assuming that the foreign cells rather than the matrix are the antigens that cause the production of hostile antibodies.

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CHAPTER 10

Transplantation of Cartilage in Animals

We are apt to assume that valuable experimental work regarding the behavior of free tissue grafts is a concomitant of twentieth century science. This is certainly not true of cartilage grafts, since observations made by some men almost a century ago have been confirmed by recent experiments. These early investigators buried fresh autogenous and homogenous grafts in a wide variety of animals and they also transplanted preserved homogenous cartilage grafts and gave accurate descriptions of the microscopic findings. It is interesting to note that the observations by Bert (1) and Prudden (2) are in agreement with our present knowledge regarding the fate of fresh autogenous and homogenous cartilage grafts in animals as well as that of preserved homogenous cartilage grafts.

FRESH AUTOGENOUS AND HOMOGENOUS CARTILAGE GRAFTS

Early Experimentation

A Frenchman, Paul Bert (1) (1865), is usually accredited with being the first to transplant cartilage in animals. Bert buried the tail of a rat subcutaneously as a free transplant and observed that nerve and muscle tissue degenerated early but that tendon, bone, and cartilage remained viable for months. The articular cartilages, which appeared to be the most durable, often became calcified and led to the formation of bone. His work was probably accomplished before 1865, which is the date of the first reference. Bert was both a physiologist and politician, being a member of the French National Assembly, where he sat on the extreme left. In harmony with his advanced attitude he advocated liberalizing national education.

Rehn and Ruef stated that cartilage grafts had been studied previously by Middeldorf (3) (1852), who reported complete absorption of all grafts. Ollier (4), working with rabbits and fowl, also noted degenerative changes in the grafts that ultimately led to their absorption. Wounds in the articular cartilage of young rabbits healed but similar wounds in adult rabbits showed no repair. *Cartilage with perichondrium* survived longer than cartilage grafts with the *perichondrium removed.*

Similar observations were made by Tizzoni (5) in 1878 and by Zahn (6) in 1884, namely, degenerative changes occurred in cartilage grafts that ultimately led to their absorption. The conclusion was drawn by Zahn that cartilage grafts degenerate whether transplanted in the same animal or in different animals.

Leopold (7) in 1881 had demonstrated survival and growth of fetal cartilage grafts

placed in the anterior chamber of the eye, in the abdomen or in the jugular vein. He was unable to demonstrate any growth in transplants of non-fetal cartilage in rabbits and concluded that ordinary cartilage grafts remained stationary, decreased in size or were eventually absorbed, whereas fetal cartilage grafts always grew even as heterotransplants.

In 1881 Prudden (2), a prominent American pathologist, buried both living and alcohol-killed cartilage grafts in rabbits. He carefully studied sections of fresh homogenous rib cartilage transplants buried in abdominal fat up to 399 days, and noted that they may remain unaffected for many months. The cells appeared viable and sometimes changed their shape and size, entering into the formation of embryonal cartilage or taking part in the formation of new connective tissue. Prudden removed all perichondrium from his cartilage grafts before making transplants and in this way he was the first to demonstrate that the grafts in animals may survive without attached perichondrium. His observations on alcohol-fixed grafts will be described under "Preserved Cartilage Grafts." In experimenting with transplanted costal cartilage, both with and without perichondrium, Fischer (8) in 1882 reported that the former survived. Contrariwise, grafts with perichondrium removed degenerated in about eight weeks. Thus, Fischer agreed with Ollier in emphasizing the importance of the attached perichondrium for the survival of cartilage transplants. He also observed that young cartilage grafts had growth capacities and that fetal cartilage grafts possessed great regenerative powers.

Summary Comment

The findings and conclusions in this early experimental work with animals appear to be somewhat conflicting and may be summarized as follows: 1) Fresh autogenous and homogenous cartilage grafts survive after transplantation either with or without perichondrium. 2) Both autografts and homografts tend to degenerate after transfer. 3) Grafts transplanted with perichondrium survive, whereas grafts without perichondrium degenerate and disappear. 4) Autografts survive but homografts tend to be absorbed. The investigators who experimented with fetal cartilage grafts noted their capacity for growth, and some perceived that young cartilage grafts had regenerative powers.

Later Experimental Work

Prominent among later investigators was von Mangoldt (9), who presented evidence that cartilage transplants survive up to nineteen months following transplantation. On the other hand, von Helferich (10) (1899) recorded absorption in his epiphyseal rabbit cartilage grafts. Others presented opposing findings, which served to confuse rather than to clarify the early experimental work.

John Staige Davis added to the knowledge of cartilage grafting by two important experiments. In 1913 he transplanted autogenous rib cartilage grafts in pedunculated flaps on dogs, and in clinical and microscopic examination observed that the grafts retained most of their bulk up to four months and appeared to contain viable cells (11). In several instances fresh costal cartilage homografts from other dogs were transplanted into the flaps and these also retained their bulk up to four months after transfer. In a study (12) of transplanted free grafts of bone and cartilage in 1917, observations were made that the cartilage remained viable up to 582 days, while the bone was almost completely absorbed. Microscopic examination showed living cartilage cells with rather normal-appearing matrix, aside from some central calcification and a moderate amount of absorption. The presence or absence of perichondrium did not affect the survival of the chondrocytes.

Confirming the relative viability of cartilage grafts reported by Davis, Lexer (13) came to the conclusion that hyaline cartilage from the ribs and joint surfaces had a remarkable tendency to survive as a free living transplant.

Mannheim and Zypkin (14) transplanted autogenous cartilage grafts into guinea pigs and concluded that the grafts retained their specific structure in most instances. They made the rather surprising statement, however, that cartilage denuded of perichondrium survived better than grafts which were transplanted with perichondrium attached.

Leo Loeb (15) undertook a long and carefully-controlled series of experiments in animals, the report of which is the finest of its kind in the literature. His important findings and views may be summarized as follows:

1. When cartilage grafts from various sources are buried as autogenous grafts there is very little reaction in the host tissues surrounding the grafts. After homotransplantation, however, lymphocytes and other cells collect in great numbers at the site of grafting. This reaction in the host tissues increases when the donor is genetically further removed from the recipient. Accordingly, the most severe reactions were seen when cartilage grafts were taken from animals of a different species (heterografts).

2. Loeb explained this host tissue reaction on the basis that the tissues of a given animal have their own special differentials which in the animal form a harmonious whole, each being chemically compatible with the others. Hence, autografts can be transplanted from one part of the animal's body to another, giving rise to very little host-tissue reaction. The specific chemical differentials of homografts and heterografts come in conflict with those of the host tissues; consequently the cellular reaction is more intense.

3. Fresh living homogenous cartilage grafts were transplanted serially by Loeb in young and old rats. It was observed that the severity of the reaction in the host tissues diminished with each successive transplantation of the original graft in the tissues of a different animal. Many of the cartilage cells remained alive up until four years and nine months. Additionally, the perichondrium produced new cartilage cells associated with the production of hyaline matrix. Fewer lymphocytes were seen in the host tissues about the grafts after each serial transplantation.

In experiments on dogs, Bisgard (16) noted that re-formation of costal cartilage by the perichondrium did not occur when cartilage ribs had been removed. When he inserted segments of rib bone in the vacant perichondrial sheath, he discovered that the bone fused together to form solid support for the chest cage.

After killing a rabbit, Kirkham (17) removed five pieces of auricular cartilage one hour after death. Two grafts in an unmoist condition and two in Ringer's solution were placed on ice. The fifth piece was transplanted into the abdominal fat of another rabbit (fresh homograft). At hourly intervals up to six hours after the rabbit's death another piece of cartilage was removed and placed in the abdominal fat of a live rabbit. At periods of twenty-four and forty-eight hours the iced cartilage grafts (dry and moist) were implanted. Six months later the grafts were removed and sectioned. All had retained their cartilaginous structure, with the exception of the graft which had been placed on ice and allowed to dry out or become desiccated. Microscopic examination showed that the cell spaces were vacuolated and filled with cellular debris. Kirkham concluded that the homogenous cartilage grafts which had been kept moist retained their cartilaginous structure even though cellular death occurred.

Experiments on dogs led Forest Young (18) to infer that autogenous rib cartilage grafts when transplanted into the subcutaneous tissues remain living and maintain their

placed in the anterior chamber of the eye, in the abdomen or in the jugular vein. He was unable to demonstrate any growth in transplants of non-fetal cartilage in rabbits and concluded that ordinary cartilage grafts remained stationary, decreased in size or were eventually absorbed, whereas fetal cartilage grafts always grew even as heterotransplants.

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tend to retain their cartilaginous structure. The chondrocytes in the grafts appear to survive as living cells, and in embryonic and young cartilage grafts the cells undergo cell division associated with the production of additional matrix, so that the statement may be made that such grafts increase in size or grow following transfer.

Not infrequently bone formation takes place in the substance of the grafts or at the periphery but this usually occurs in small portions of the matrix, so that the greater bulk remains as cartilage.

Adult grafts tend to retain their same general size but occasional grafts may be invaded and absorbed.

Grafts survive equally well whether transplanted with or without perichondrium.

In his interesting work with dogs Bisgard noted little regenerative or reparative tendency when the rib cartilages were removed though the perichondrial sheath remained in place. Bone segments placed in the perichondrial sheaths fused together to form solid rib structures, which suggested a clinical use in humans when large amounts of cartilage are removed from the chest cage. Chips or segments of bone from the ilium might be seeded in the perichondrial sheaths from which cartilage has been removed.

Fresh Homogenous Cartilage Grafts

This type of graft appears to be tolerated quite well in animal tissues for long periods of time. Loeb noted a more intense cellular reaction in the host tissue surrounding homografts than in the tissue surrounding autografts. He also found a greater tendency to invasion and absorption in homografts.

Dupertuis observed that chondrocytes in young homogenous cartilage grafts in the rabbit not only survived but also multiplied or at any rate formed additional matrix so the homografts actually increased in size. Young also believed that the cartilage cells in his experiments on dogs remained viable but that the grafts retained their same general size up to 1½ years. Bacsich and Wyburn noted that chondrocytes in fresh homogenous grafts survived transplantation and showed evidence of actual proliferation.

Thus, the more recent experimental work with fresh homografts of cartilage confirms the early findings of Prudden that the cells in the grafts survive, that the matrix appears to remain as cartilaginous intercellular material in many grafts, and that active proliferation of the cartilage cells may occur.

While other investigators had reported occasional new cartilage formation by the cells in fresh homografts, Dupertuis was the first to take accurate measurements and demonstrate orderly and consecutive growth in young cartilage grafts. This he presented in a very fine paper, which contains additional observations not listed in this summary. *The growth property, however, does not appear to be present in human homocartilage, or at any rate it is not as active, as will be demonstrated later.*

Apparently, in lower animals the distinction between autogenous and homogenous is not as definite as in man. The higher we go in the animal scale the more specialized and less adaptable do the tissues become. Many tissues which can be successfully transplanted as fresh homografts in lower forms will not survive or survive as long in man under similar circumstances. Embryonic tissues which can be successfully cross-grafted lose this flexible characteristic as they develop and become more specialized.

TRANSPLANTATION OF DEAD CARTILAGE GRAFTS IN ANIMALS

The American, Prudden (2) (1881), is considered as the first to transplant preserved dead cartilage as homografts in animals. He buried grafts fixed in alcohol or phenol, or treated by electric desiccation, boiling or

size and weight for as long as one and a half years. About the same time Dupertuis (19) (1941) buried measured segments of rabbit-ear and rib-cartilage grafts in the same rabbits and in different rabbits. The autografts grew or increased in size 122 per cent over a period of 161 days. The homografts increased 56 per cent over a period of 173 days. The cells in these fresh homografts appeared to be viable but some grafts showed bone formation in the matrix and outside the grafts. Fresh adult cartilage buried in young rabbits showed no evidence of growth, but young cartilage transplanted into the tissues of adult rabbits grew exceedingly well. A homogenous piece of young perichondrium showed new cartilage formation on removal.

In later experiments on dogs Young (20) (1945) transplanted 58 fresh (living) homografts into the abdominal fat of ten dogs and removed the grafts at intervals of two weeks to 1½ years. All of the grafts retained their same general size but degenerative changes were present in the cells and ossification occurred in the matrix of some grafts. Young believed that his grafts were viable.

Bacsich and Wyburn (21) (1947) noted that fresh cartilage homografts transferred to the subcutaneous tissues of guinea pigs tend to preserve their original shape, size and form for some time and histologically appear as normal cartilage. The matrix remains unchanged and there is evidence of active proliferation of the cartilage cells (viable) with amitotic division of chondrocytes. There was no encapsulation but some evidence of a mild host reaction in the form of lymphocytosis and fibroblastic proliferation (22).

Fresh iliac bone and cartilage were transplanted by Hutchison (23) (1949) into the anterior chamber of the eye of rabbits. Grafts from the same rabbit and from different rabbits were removed and examined at intervals of ten days to 180 days. The cartilage retained its cartilaginous structure and the chondrocytes appeared as living cells in both homotransplants and autotransplants.

Cartilage was not produced by injections of various substances into the muscle of rats, as reported by Kathryn Stephenson (24) (1952). These substances were 0.1 N hydrochloric acid, 40 per cent alcohol, acidified 40 per cent alcohol, phosphatase, 40 per cent alcoholic extract of cartilage, benzene extract of cartilage, hyaluronic acid and purified chondroitin. Her studies indicate that the role of various lipid fractions of cartilage with regard to new cartilage formation (chondrogenesis) should be further investigated.

Wagenfeld (25) (1952) transplanted homogenous ear cartilage into adult rats and made observations on more than 300 specimens. After 24 hours the inner layers of perichondrium loosened and the cells assumed a round form. These cells divided rapidly by mitosis and on the second day exceeded the thickness of normal perichondrium. On the fourth day a capsule began to form in the inner part of the chondrogenic layer of cells. Through further transformation of perichondrial cells the young cartilage cells increased and formed nests of nodular cartilage. Soon reaching a large size, these cartilage nests were separated by fine connective-tissue septa.

Summary Comment

While much of the earlier experimental work is conflicting, later more carefully controlled observations confirmed some of the earlier findings and in certain instances established new facts.

The available data at this time led to the following conclusions:

Fresh Autogenous Cartilage Grafts

Living autogenous grafts from various donor sites, with or without perichondrium,

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drying. His results showed degeneration and partial absorption of all these grafts.

Seggel (26) in 1904 had noted early destruction of alcohol-preserved grafts, and Nageotte (27) found that rabbit-ear cartilage preserved in alcohol was subject to invasion and absorption following transfer. He noted a tendency to new formation of cartilage and bone in and about his grafts.

Polettini (28), Nigrisoli (29) and Didier and Guyon (30) transplanted dead cartilage homografts from guinea pigs, rabbits, and calves. Their findings of invasion of the grafts often associated with new cartilage and bone formation agreed with those of Nageotte.

Dupertuis transplanted cartilage grafts preserved by various methods (with dead cells) into rabbits. These grafts included autografts, homografts, and heterografts (preserved dog cartilage). He removed the preserved autografts and homografts after an average period of 232 days; every graft showed gross absorption and reduction in size. All sections had dead degenerating cartilage with no living cells or areas of regeneration. In most instances there were only fragments of pale ghost-like cartilage structure, and fibrous tissue actually invaded these portions. Several areas of calcification were present and early bone formation was observed in one section. Rib cartilage appeared to resist invasion rather better than grafts of elastic ear cartilage. Examination of the preserved heterografts confirmed Leo Loeb's findings in that they produced a more intense reaction in the host tissues than the fixed autografts and homografts.

Laskin and Sarnat (31) investigated the metabolic activity of fresh rabbit cartilage, preserved (frozen-dried, in merthiosaline) cartilage autografts, and fresh and preserved cartilage homografts. The rate of respiration and anaerobic glycolysis of control fresh rabbit costal cartilage were among the lowest of all tissues. No metabolic activity was recorded after the tissue had been autoclaved. During the first seven days following transplantation the rates of respiration and anaerobic glycolysis in both the autografts and living homografts decreased to almost half that of fresh control cartilage. After this initial decline no further significant variations occurred in either series. Cartilage preserved in merthiosaline or by freezing-drying for seven days exhibited only about one fifth the normal rate of carbohydrate metabolism.

Comment

The general opinion regarding the fate of preserved homogenous cartilage grafts transplanted in animal tissues is that the cartilage tends to be invaded and absorbed. Some observers have noted bone formation in the cartilage, and a few have reported new cartilage formation.

The meager reports on preserved heterografts indicate absorption preceded by a more intense cellular reaction in the host tissues.

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ficiently protected by gel-like mucoprotein, the cells are destroyed in short periods of time presumably by the hostile host antibodies (1). The heterograft because of its foreign nature fares even worse than the homograft.

Thus, it appears that the tissue-specificity theory of Leo Loeb (3) and the antibody theory of Medawar may fit in very neatly with the mucoprotein-protective hypothesis proposed by Bacsich and Riddell (2) to explain the long survival time of cartilage homografts. Loeb conceived of transplantation immunity as a local reaction, whereas Medawar believes that transplantation immunity is the result of a systemic and not a local reaction.

Wyburn (4) suggests that the mucoprotein in the matrix of homogenous cartilage grafts may interfere locally with the defense mechanism of the host and may act either by neutralizing the tissue antigens of the homograft or may afford some protection against host antibodies as in encapsulated pneumococci. The capsule of the pneumococcus is rich in mucopolysaccharides and the bacterium protects itself at will by increasing the thickness of its capsule in the presence of hostile host antibodies. One may regard the pneumococcus in human tissues as a fresh heterograft with its own special method of combating the hostile host environment.

Experimental work with cartilage grafts in humans is not extensive, and the reports are relatively recent in comparison with those on animal experimentation. The early publications and most of the later ones deal with clinical evaluation rather than experimental studies. This is quite understandable, since experimental work in humans is associated with numerous difficulties, including the danger of legal suit.

In this chapter both clinical and experimental articles will be presented in chronological order to include all available evidence regarding the fate of cartilage grafts in humans.

FRESH AUTOGENOUS AND HOMOGENOUS CARTILAGE GRAFTS

Pioneer Work

König (5) in 1896 was possibly the first to use fresh cartilage transplants in man, though it is quite likely that other surgeons employed this type of graft earlier. He buried segments of autogenous rib cartilage as wedges for repair of partial destruction of the laryngeal and tracheal cartilages. Von Mangoldt (6) in 1899 successfully transplanted autogenous costal cartilage into the nose. Nélaton and Ombredanne (7) in 1904 buried autogenous costal cartilage in forehead skin flaps, which were later swung down to the nose.

In 1911 Tuffier (8) reimplanted the head of the humerus with its articular surface after resection of its upper end. The functional result was excellent but the persistence of the cartilage as such was not demonstrated. Lexer (9) in 1907 reported his well-known case in which he transplanted the upper third of the tibia with its articular surface in the fresh state from an amputated limb as a living homograft to replace a loss in another patient. The clinical result was reported to have been good but again persistence of the cartilage was not demonstrated.

Staige Davis (10) in 1917 gave clinical evidence of the durability of autogenous rib cartilage transplants in the nose. Gillies (11) in 1920 stated that no clinical changes other than curvature were observed in any of his successful fresh autocartilage grafts, and in only a few of the fresh homografts was the cartilage reduced in size or replaced as a late sequel. These grafts were observed *in situ* up to three years following transfer. Gillies was the first investigator actually to remove a fresh human cartilage autograft and homo-

CHAPTER 11

Transplantation of Cartilage in Humans

One should use great discretion *in applying the results of animal experiments with cartilage grafting to the behavior of cartilage grafts in the human.* Two examples of this difference are as follows.

Fresh young autogenous and even homogenous cartilage grafts tend to increase in size or grow in the rabbit (Dupertuis), whereas in the human, similar grafts do not tend to grow.[1] Indeed, in the human, fresh homografts not only fail to grow but are slowly (and sometimes rapidly) reduced in size through the activity of host fibroblasts which invade and replace the cartilage matrix. The chondrocytes remain viable until their protective armor of matrix is removed and they are exposed to hostile host cells, host antibodies or some other host elements. They then disappear in the great multitude of invading fibroblasts, and it is probable that the exposed chondrocytes are destroyed like the epidermal cells in homogenous skin grafts.

In animals, absorption of cartilage grafts followed by new cartilage formation has been observed by a number of careful investigators. In humans, absorption of fresh autogenous and homogenous grafts is known to occur, *but no accredited investigator has ever reported the new formation of cartilage in or about a human cartilage graft buried in human tissues.* Partial absorption of human cartilage grafts and replacement by new bone formation, however, are not infrequent. One must also be critical in applying the results of tissue culture work and the behavior of cartilage grafts in ideal transplantation sites like the anterior chamber of the eye to the fate of cartilage grafts *in vivo* and in normal host beds.

Most experienced investigators now accept the belief that fresh autogenous grafts are always preferable to fresh, preserved or frozen homogenous grafts as grafting material. Fresh or preserved homogenous cartilage grafts are used as a second choice when it is not expedient to utilize the patient's own cartilage.

In general, foreign grafts are believed to stimulate an immune response in the host tissues, which is detrimental to the survival of various homogenous tissue grafts over various periods of time, as demonstrated by Medawar (1). Fresh homogenous grafts of cornea or lens and fresh homogenous cartilage grafts retain their structure for long periods of time because they are avascular tissues and because their gel-like matrix is rich in mucoprotein, which serves to protect the cells from hostile host antibodies (2).

Alternately, in tissues such as homogenous surface skin grafts and buried homogenous fat grafts, which are vascular and not suf-

[1] Unpublished experimental work of the author.

tilage grafts. Some felt that these grafts remained as cartilage, while others believed that the grafts were slowly replaced by fibrous tissue. A few surgeons had given up the use of rib cartilage grafts entirely, preferring bone grafts from ribs, tibia or ilium. Kazanjian was employing autogenous bone grafts from the tibia in preference to cartilage grafts.

The opinions regarding autogenous septal cartilage were unanimous; those who answered the questionnaire agreeing that since the grafts were always absorbed they had no place in clinical surgery. This seems rather odd, for septal cartilage grafts had been used clinically, both as fresh autografts and as homografts preserved in alcohol, by many otolaryngologists to fill small saddle depressions in the nose. Possibly the high incidence of infection which often occurred discouraged their use.

PRESERVED HOMOGENOUS CARTILAGE GRAFTS

During the early days of otolaryngology physicians desiring to become specialists in this developing field attended postgraduate clinics both here and abroad. After observing the operation of submucous resection for a few weeks the student was permitted to do this procedure on some derelict patient while he sat up in a chair, under the beneficent effect of cocaine anesthesia. Naturally the student's first few patients usually developed a saddle depression of the nose. This was so common an occurrence that the clinic chief very wisely instructed his assistants to preserve the cartilage removed at operation in bottles of alcohol. Thus, saddle deformities arising through a student's early operations could later be repaired by inserting segments of preserved septal cartilage beneath the skin overlying the depression.

It is curious that, although preserved human septal cartilage was often used as a free graft in many outpatient clinics, no written records appear in the literature describing the procedure. This method, which was widely used, fell into disrepute because of the high incidence of infection and the clinical belief (expressed by Joseph Beck and others) that the grafts were always absorbed and replaced by fibrous tissue (both autogenous and preserved homogenous grafts).

O'Connor and Pierce (13) in 1938 reported the clinical use of rib cartilage preserved in physiologic solutions of sodium chloride and merthiolate (4:1) and kept at refrigerator temperature. Three hundred and seventy-five preserved homografts observed clinically over a period of five years showed no evidence of absorption and less tendency to bend and curl when compared with autografts. The preserved homografts also had great ability to resist infection, and the graft structure was not always lost when infection did occur provided early drainage was instituted. It should be emphasized that O'Connor and Pierce had been using these preserved rib-cartilage homografts for some time before their important article on the subject appeared in 1938. Visiting plastic surgeons were intrigued by the possibilities of these preserved homografts, data which Pierce and O'Connor discussed in select circles but were reluctant to publish until they were sure that the grafts were of real clinical value. Thus, Claire Straith and other plastic surgeons were utilizing preserved human rib-cartilage grafts clinically before the original data were published.

Gillies (14) in 1937 had presented cases in which he constructed a new external ear using maternal ear cartilage as the structural support (fresh homogenous cartilage). From a clinical standpoint the transplanted maternal cartilage appeared to retain its structure as a support for the contour of the auricle.

Peer (15) in 1938 reported his findings from the microscopic examination of transplanted sections of human septal cartilage

graft and report the microscopic findings. Both grafts were removed after 18 months and on section showed the cartilage cells alive and active in both instances. The cells in the homograft were more vacuolated and showed more degenerative changes than did those of the autograft. The autograft appeared as normal adult cartilage.

Neuhof (12) in 1923 made an extensive survey of the literature on cartilage transplantation in animals, and a clinical evaluation of fresh autogenous and homogenous cartilage grafts in humans. Neuhof did not distinguish clearly between fresh autografts and fresh homografts. Furthermore, no mention was made of the microscopic examination of a fresh autogenous and homogenous graft, as reported by Gillies in 1920.

From his survey Neuhof came to certain conclusions regarding the fate of fresh cartilage grafts transplanted in humans. Simple[2] cartilage grafts after transplantation remain unaltered in appearance and staining reaction for many weeks. Only in grafts that are several months old does fibrillation of the cartilage begin. The cartilage cells undergo gradual death and ultimately disappear. Vascularization of the graft and replacement by fibrous tissue or calcification may occur, depending on the locality to which the graft is transferred. The outstanding feature in the histologic fate of cartilage transplants is the long period of quiescence that precedes the final phase of degeneration and substitution.

Questionnaire Opinions on Behavior of Cartilage Grafts in Humans

In 1934 a questionnaire was sent by the author to fifteen plastic surgeons with wide experience in cartilage grafting, asking for an opinion, either clinical or experimental, on the behavior of autogenous human cartilage grafts of all varieties (rib, septal, alar and ear cartilages).

[2] Simple probably means free cartilage grafts.

The opinions expressed in response to these letters dealt mainly with rib and septal cartilage. None of the surgeons replying had actually removed grafts and examined them microscopically, with the exception of Gillies, who had investigated and examined one fresh rib autograft and one fresh homograft in this manner. On the basis of this examination and his comprehensive clinical experience Gillies stated that rib cartilage grafts, especially the autogenous variety, survived after transfer and retained their same general size regardless of the presence or absence of perichondrium.

Staige Davis was more cautious and gave as his view that the presence of perichondrium aided in the survival of autogenous cartilage grafts. He had not examined any human grafts microscopically so he based his conclusions on clinical evaluation and animal experimental work.

Warren Davis believed that autogenous rib cartilage grafts tended to remain unaffected; and Sheehan stated characteristically that "morsels of fresh costal and alar cartilage survive very well indeed." Sheehan did not favor the use of septal cartilage grafts, which he felt were absorbed in most instances.

Dorrance and five other surgeons expressed the opinion that human autogenous cartilage grafts were very slowly replaced by fibrous tissue, which often maintained the architecture of the graft. They did not favor the use of septal cartilage grafts.

Joseph Beck, a prominent otolaryngologist and pathologist of Chicago, gave his view that septal cartilage grafts were always absorbed after transplantation and that they had no place in plastic surgery. He also believed that rib cartilage grafts, while very resistant, were slowly replaced by fibrous tissue.

In summarizing these clinical opinions expressed in 1934 it is evident that the majority of plastic surgeons used autogenous rib car-

tilage grafts. Some felt that these grafts remained as cartilage, while others believed that the grafts were slowly replaced by fibrous tissue. A few surgeons had given up the use of rib cartilage grafts entirely, preferring bone grafts from ribs, tibia or ilium. Kazanjian was employing autogenous bone grafts from the tibia in preference to cartilage grafts.

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homografts preserved in 50 per cent alcohol. These grafts had been transplanted beneath the chest skin of other humans and removed at intervals of from seven days to 14 months. A reaction of the foreign-body type was seen in the host tissues about the transplant. This reaction lasted until the thirty-second day after transplantation and was largely absent in sections buried for a longer time. From 32 days through four months the grafts remained as tolerated dead foreign bodies. In the section buried for 14 months there was considerable absorption of the cartilage matrix and in one area true bone formation had occurred.

In 1939 Peer (16) described the microscopic findings in living autogenous rib grafts and preserved homogenous rib and septal cartilage grafts buried in humans. The dead preserved cartilage grafts showed progressive invasion and partial absorption. In contrast to these findings the autogenous rib cartilage grafts buried up to six years showed no evidence of invasion or absorption. The chondrocytes in the autografts, which were all buried without perichondrium, appeared as living cells.

LATER OBSERVATIONS ON HUMAN CARTILAGE GRAFTS

Barrett Brown (17) in 1940 summarized his observations made on preserved (in alcohol, by refrigeration and freezing) and fresh cartilage homotransplants over a period of eleven years. In clinical agreement with many other plastic surgeons, he believed that the preserved homograft was a second-choice substitution transplant. Most of the homogenous cartilage used was from cadavers. In his opinion human costal cartilage appears to be somewhat unique in that its exact counterpart in easily available animals has not been found. Patients have retained preserved cartilage transplants in the nose for as long as three years. Other areas in which this type of cartilage graft was used were cheeks, chin, jaws, orbit, forehead and temporomandibular joint.

As viewed by O'Connor (18) in 1940, the cartilage homograft may be employed regardless of race, sex, color or blood grouping. He has used 400 refrigerated homogenous cartilage grafts, including septal, ear, articular and sections of costal cartilage, in a period of over six years to correct bony or cartilaginous defects of the head, face, chin, nose and orbital rim, and in ear reconstruction. All but one have retained their original identity and have not been absorbed. He believes that these grafts curl and bend but much less frequently than fresh autogenous cartilage grafts. In his hands the cartilage homograft has entirely replaced the autograft.

From clinical observation and occasional removal of grafts which had become distorted or hoarded as excess cartilage beneath the abdominal skin, Mowlem (19) in 1941 concluded that there is no clinical difference between the autograft and the homograft.[3] Fresh homogenous maternal ear cartilage as well as fresh homogenous rib cartilage survive well in their host beds, and this does not appear to depend on blood grouping. Mowlem noted that in many cases a graft removed after a period as long as five years following insertion still bears the original knife markings. It is embedded in a layer of dense fibrous tissue which replaces its perichondrium and which in some patients invades and pits the surface to a depth of perhaps half a millimeter.

Prior to 1941, both otolaryngologists and plastic surgeons believed that fresh autogenous septal cartilage grafts were absorbed shortly after transplantation and therefore should not be used as grafting material. This belief was not in accord with Peer's (20) clinical experience and he therefore buried autogenous septal cartilage without

[3] At the present time (1954) Mowlem prefers to use autogenous cartilage grafts. Personal communication.

perichondrium beneath the abdominal skin of patients and removed the grafts at intervals of from two weeks to three years for microscopic examination. He found that the septal cartilage grafts retained their structure and that the chondrocytes survived transplantation as living cells. All of the septal cartilage grafts had about the same bulk when removed as when first transplanted. He concluded that both rib and septal cartilage grafts retain their hyaline structure after transplantation as autografts in humans and that the chondrocytes remain viable.

The gelatinous intercellular substance of cartilage is one of the most durable of all intercellular substances. Following transplantation cartilage may even persist for long periods of time as an autogenous graft when the cells in the graft have been previously killed by heat or other agencies. Gordon New (21) made practical use of this physiological fact when he advocated the use of "boiled" autogenous cartilage grafts, which have less tendency to bend or become distorted following transplantation. New reported a series of cases in which he had used heat-treated autogenous rib cartilage as grafting material with excellent clinical results. He commented on the durability of the graft structure, which represented a non-living autogenous graft.

Straith and Slaughter (22) in 1941 reported on 100 cases in which homogenous rib cartilage preserved in merthiosaline had been used to restore facial contours. Eighty one per cent were without complications and 94 per cent were satisfactory in spite of complications. Straith and Slaughter concluded: In view of the biologic rationale and the encouraging results obtained in actual practice, it seems logical to accept the use of homocartilage preserved in "merthiosaline" as the basis on which to restore facial contour. Homocartilage has the great advantage of eliminating the somewhat hazardous operation of costal resection with its prolonged incapacity and morbidity. Straith was among the first to use preserved homocartilage clinically as a routine procedure. His fine results stimulated the author to study the graft experimentally.

Greeley (23) in 1941 had reported several completed ear reconstructions, in which he had used Gillies' fresh maternal ear cartilage method, at a meeting of the American Society of Plastic and Reconstructive Surgery in New Orleans (1944). Greeley stated in a discussion that the cartilage in his patients was absorbed.

Gillies (24) in 1942 presented sixteen cases of external ear reconstruction in which fresh maternal ear cartilage had been used for structural support. In his opinion the grafted cartilage retained its structure following transplantation insofar as could be determined by appearance and external palpation.

An interesting study of the structure of cartilage was made by Bucher (25) in 1942. Hyaline cartilage arises from two constructive elements: "chondrones," and fibrillar processes running between them. The chondrones are cartilage cells, around which collagen fibrils are spun like a shell; functionally these cells operate as elastic traction bodies. All collagen elements of hyaline cartilage are embedded in the chondromucoid, which is gradually lost with the transition into perichondrium. Bucher worked with tracheal, laryngeal, nasal and rib cartilage in adult humans, tracheal cartilage of horses, cattle, dog and rabbit, and also cartilage from a Scyllum cancicula. All of these cartilages showed the architecture of hyaline cartilage to be comprised of both an oblique, S-forming and a criss-cross arrangement of the fibrillar systems. This seemed to be the structural principle according to which hyaline cartilage in man and animals is built.

In a paper describing the formation and use of diced cartilage grafts in 1943, Peer (26) reported the microscopic and gross

changes occurring in and about the numerous cartilage segments. Diced costal cartilage grafts were stored beneath the chest skin of seven patients. After successful operative repair of the skull depression or other defect was assured, the stored excess diced cartilage grafts under the chest skin were removed at intervals and sectioned. Examination showed that the spaces between the separate cartilage segments were occupied first by blood and later by ingrowing connective tissue accompanied by numerous blood vessels. After the ingrowing fibroblasts had elaborated collagenous fibers, contracture occurred binding the mass of diced cartilage grafts into a solid plaque. Autogenous diced rib-cartilage grafts showed living chondrocytes and normal-appearing matrix with an absence of invasion or absorption. Control sections of preserved homogenous diced cartilage grafts showed definite invasion, partial absorption of the cartilage and occasional bone formation. Grafts were examined in both instances up until three years after transplantation.

In a discussion of the reconstruction of the absent ear, Lamont (27) in 1944 used the term "necrocartilage" to describe cartilage removed from cadavers. He placed one piece of this type of ear cartilage in merthiolate solution and another piece in an abdominal wall pocket; after 96 days when both were studied microscopically the specimen in the solution appeared better preserved. The one in the abdominal pocket had not decreased in size but much of it had been replaced by fibrous tissue. Ear and rib cadaver cartilage were compared with fresh autogenous ear cartilage. Again sections of each kept in merthiolate solution were in better condition than the sections placed in abdominal wall pockets. All of the latter were invaded by fibrous tissue. Then sections of autogenous rib cartilage placed in the nose and abdominal wall of a patient were examined microscopically at the end of 272 days, when no difference in them could be noted. This tends to refute the statement made by some writers to the effect that cartilage transplanted into the nose is in a totally different medium from that placed beneath the abdominal wall.

Ivy (28) (1944) expressed preference for costal cartilage because it rarely undergoes absorption when embedded in soft tissues and is not as susceptible to infection as bone. In his opinion, living autogenous costal cartilage is undoubtedly preferable to any other but costal cartilage does not form a firm union with bone; hence it cannot effect a stable restoration of continuity in a movable bone. For filling gaps to restore the continuity of such bones he thinks bone transplants alone are satisfactory.

In 1944 Peer (29) summarized his experimental observations regarding the behavior of autogenous and homogenous cartilage grafting in humans. Autogenous rib-cartilage grafts with perichondrium removed retain their normal structure with living chondrocytes up to 13 years following transplantation. Autogenous septal,[4] alar and auricular cartilage grafts also tend to retain their matrix structure with living chondrocytes. The autogenous rib, septal and alar grafts also retain the specific structure of their matrix as hyaline cartilage. The elastic ear cartilage grafts retain the specific structure of their matrix as elastic cartilage. By way of contrast, preserved homogenous rib and septal cartilage grafts tend to be invaded by the surrounding host connective tissue and partly absorbed. Bone formation may occur in any cartilage graft but is more common in and about preserved homogenous cartilage grafts. Microscopic examination of a fresh homogenous human rib cartilage graft buried for three years and nine months showed complete absence of absorption and

[4] Autogenous septal bone grafts transplanted with the septal cartilage in chest fat retained their calcified structure with living bone cells up until five years after transplantation.

normal-appearing cartilage cells (apparently living). Examination of other fresh homogenous rib grafts buried for a shorter period of time showed invasion and partial absorption of the grafts but the remaining chondrocytes appeared to be viable. Peer reported the use of diced cartilage grafts in a perforated vitallium ear mold, demonstrating the physiological fact that connective tissue appears to abhor an unlined dead space as nature abhors a vacuum. He predicted that the principle might be applied to utilize diced cartilage grafts for the repair of recurrent hernia, the closure of large bony defects in spina bifida and for ankylosed joint surfaces.

On the basis of histological examination of additional autogenous cartilage grafts in humans, Peer (30) stated that autogenous septal, alar, lateral, auricular and rib cartilage all tend to survive after transplantation as living cartilage. Experimental observations indicated evidence of actual growth in some young autogenous cartilage grafts.

In 1946 Peer (31) presented additional evidence indicating that young human autografts tend to increase in size following transplantation.[5] A method for examining cartilage grafts in the fresh unfixed state was described. A human rib autograft buried for 25 years was sectioned in the fresh unfixed state and the cartilage cells took supravital dyes as do living tissue cells. Measurements of auricles reconstructed with autogenous diced cartilage grafts preformed in a vitallium ear mold failed to give any evidence of growth (32).

Padgett and Stephenson (33) in 1948 described the microscopic findings in two autogenous costal cartilage grafts buried in a patient's abdominal fat and under the forehead skin for 13 years and 8 years respectively. Both of these grafts had normal-appearing cartilage matrix. The cartilage cells appeared to be viable in the fixed and stained sections and there was an absence of invasion and absorption.

Most hospitals with plastic services have a preserved cartilage bank, as described by Barrett Brown (34). Cartilage obtained at fresh autopsies is refrigerated (frozen if possible), all soft tissue including the perichondrium removed, and again refrigerated in a solution of aqueous merthiolate.

In a case reported by Brunner (35) in 1949 the living rib cartilage had been implanted in the back of the nose as well as in the columella and after a lapse of 21 years the cartilage at the dorsum was broken up, whereas the cartilage in the columella preserved its gross appearance. In his opinion, the resulting deformity may be hypothetically explained by the influence which the surrounding tissue exerts upon the implant. If connective tissue invades the cartilage, the ground substance is resorbed more rapidly than the cells. This case, he holds, indicates that the fate of the transplanted living rib cartilage is not determined by the tendency toward degeneration but by the resistance of the cartilage against the surrounding connective tissue. Cartilage covered with perichondrium is more resistant than cartilage without perichondrium.

In 1949 Bossi (36) reported that the transplantation of autogenous hyaline cartilage ends with early destruction of the cellular elements and a successive diminution of the affinity of the basic structure for dye stains. The collagenous fibrils become evident because of the maceration and atrophy of the cement base.

A fresh human auricular-cartilage homograft which had been buried for 15 months was removed by Kazanjian and Converse (37) in 1949. The graft removed from the patient's father and transplanted to give

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changes occurring in and about the numerous cartilage segments. Diced costal cartilage grafts were stored beneath the chest skin of seven patients. After successful operative repair of the skull depression or other defect was assured, the stored excess diced cartilage grafts under the chest skin were removed at intervals and sectioned. Examination showed that the spaces between the separate cartilage segments were occupied first by blood and later by ingrowing connective tissue accompanied by numerous blood vessels. After the ingrowing fibroblasts had elaborated collagenous fibers, contracture occurred binding the mass of diced cartilage grafts into a solid plaque. Autogenous diced rib-cartilage grafts showed living chondrocytes and normal-appearing matrix with an absence of invasion or absorption. Control sections of preserved homogenous diced cartilage grafts showed definite invasion, partial absorption of the cartilage and occasional bone formation. Grafts were examined in both instances up until three years after transplantation.

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support for a reconstruction of the ear became softened and failed to give proper support. Microscopic examination showed invasion of the periphery of the graft by host connective tissue and degenerative change in the nuclei of the cartilage cells.

Dupertuis (38) in 1950 presented clinical evidence which indicated measurable growth in young human cartilage autografts over a period of four to six years. In one case he exposed rib cartilage grafts and his measurements demonstrated increases of 0.5 and 0.6 cm. in two rib segments buried for four years. The rounded ends showed a loss of sharp-cut corners, and Dupertuis interpreted this as evidence of growth activity. Measurements of rib cartilage grafts made by means of their contour projections in the overlying skin demonstrated an increase in size in each instance. It was also evidenced that the presence of perichondrium was not necessary for growth. In patients with depressed or underdeveloped malar bone and saddle deformities of the nose repaired with autogenous rib cartilage the grafts appeared clinically to increase in size with the general growth of the facial structure.

In 1951 Peer and Walker (39) reported conclusions drawn from microscopic study of 118 autogenous human cartilage grafts of various types buried in contact with unlike tissue and 20 autogenous cartilage grafts buried in contact with cartilage. The matrix of the grafts tends to retain its structure and the chondrocytes survive as living cells under both conditions of transfer. Autogenous cartilage in contact with like tissue is joined to the host cartilage by fibrous tissue and not by cartilaginous union in the infant, the young child and the adult. The growth principle appears to be absent in young human cartilage autografts regardless of the presence or absence of perichondrium. The statement that autogenous cartilage grafts retain their specific structure following transplantation is somewhat relative. Thus, portions of a cartilage graft may be replaced by connective tissue or bone, and new blood vessels may grow into an occasional graft associated with some absorption. These occasional variations do not affect the validity of the statement that autogenous cartilage grafts tend to survive and retain their structure. The latter statement describes the behavior of the majority of autogenous cartilage grafts. Autogenous cartilage grafts, unlike bone grafts, appear to survive equally well whether subject to forces of funtional use or in transplantation sites where they lie idle and have no functional use.

In an unpublished experiment Peer in 1952 removed a fresh rib cartilage homograft transplanted from an infant into the abdominal fat of a six-year-old child. The graft, without perichondrium, had been carefully measured before transplantation and was removed four years after transfer. Measurement at the time of removal demonstrated definite reduction in size. The graft was sectioned in the fresh unfixed state and microscopic examination showed that all of the cartilage cells completely filled their lacunal chambers and appeared as living cells. (This was confirmed by staining with dilute supravital dyes.) In one area of the graft considerable absorption had occurred, and this area of absorption was occupied by young plump fibroblasts accompanied by numerous small blood vessels. No host cells other than the fibroblasts were seen. Chondrocytes protected by only a thin armor of matrix were living cells but no free cartilage cells were observed in areas where the matrix had been completely removed. The findings in this homogenous cartilage graft support the theory of Bacsich and Riddell (2) that the mucoprotein in cartilage protects the foreign chondrocytes against hostile host antibodies. When the protective matrix is removed (presumably by host fibroblasts)

the unprotected chondrocytes are destroyed, or at any rate they disappear.

Preserved interarticular disc cartilage was used by S. Vidaurre (40) of Chile in four cases of nasal reconstruction with apparent success, and this type of graft was utilized clinically by Mir y Mir (41) for defects in the chin, nose and ear. The latter buried preserved homogenous meniscus-cartilage grafts beneath the muscular aponeurosis in the arm and thigh of patients and removed them for histologic examination in 7 and 14 months after transplantation. He saw no appreciable change in the size or structure of the preserved fibrocartilage grafts, which appeared to retain their elasticity and have less tendency to absorption than preserved rib-cartilage grafts. Stanley Barkus (42) has also used preserved meniscus cartilage in ear reconstructions, and he believes that the material as a homograft retains its structure well following transfer.

Schofield (43) recently (1953) conducted follow-up examination on 58 patients who had preserved homogenous cartilage grafts in various transplantation sites. On the basis of clinical evaluation about 50 per cent of the implants showed probable evidence of absorption at the end of two years, which suggested that further surgery may become necessary in many cases after five years. Schofield advocates the use of preserved cartilage implants for repairing contour defects in children, whose growth makes necessary the introduction of repeated implants. The grafts were preserved in merthiolate,[6] 1:4000, in normal saline.

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CHAPTER 12

Transplantation of Cartilage in Humans (Continued)

SUMMARY COMMENT

Autogenous Cartilage Grafts

Apparently all kinds of autogenous cartilage can be successfully transferred to the human as free grafts. In most instances the chondrocytes survive as living cells which continue to service and maintain their cartilaginous matrix. Hyaline matrix remains hyalin, elastic intercellular substance retains its specific structure as elastic cartilage, and fibrocartilage probably remains as fibrocartilage.

The bulk of the grafts is usually retained following transfer but occasional variations do occur, and some grafts may be considerably reduced in size due to invasion and replacement by host connective tissue, which does not maintain the architecture of the graft. When bone replacement takes place the architecture of the graft tends to be retained, and the new bone is always supplied by blood vessels rather than by diffusion of tissue fluids. The latter circulatory mechanism, however, continues to supply the cartilage, which refuses to accept the more modern vascular supply system. Thus cartilage resembles the epidermis of skin since epidermis also retains its non-vascular type of circulation under all conditions of transplantation.

A cartilage graft in contact with cartilage, bone, or any other host tissue heals by fibrous tissue union rather than by cartilaginous union, and new formation of cartilage has not been observed in human cartilage grafts regardless of the presence or absence of perichondrium or the youth of the graft and that of the recipient. Autogenous cartilage grafts in avascular transplantation sites tend to be replaced by host fibrous tissue, and occasional individual patients have a tendency to absorption of grafts even though the transplantation sites are favorable. Invasion and absorption of cartilage grafts occur more frequently in young children than in older individuals. Autogenous cartilage grafts, like all other free grafts, may fail to survive when transplanted into debilitated patients with low plasma protein, secondary anemia, diabetes or other deficiencies.

In total ear reconstruction the rib cartilage utilized to form the structural support of the auricle eventually protrudes in space and is covered by rather thin skin layers on both of its surfaces. In children, especially, gradual reduction in size of the cartilage framework may occur, and if the surgeon attempts to carve out fine details in the cartilage structure, absorption will almost always take place. It is therefore expedient to wait until

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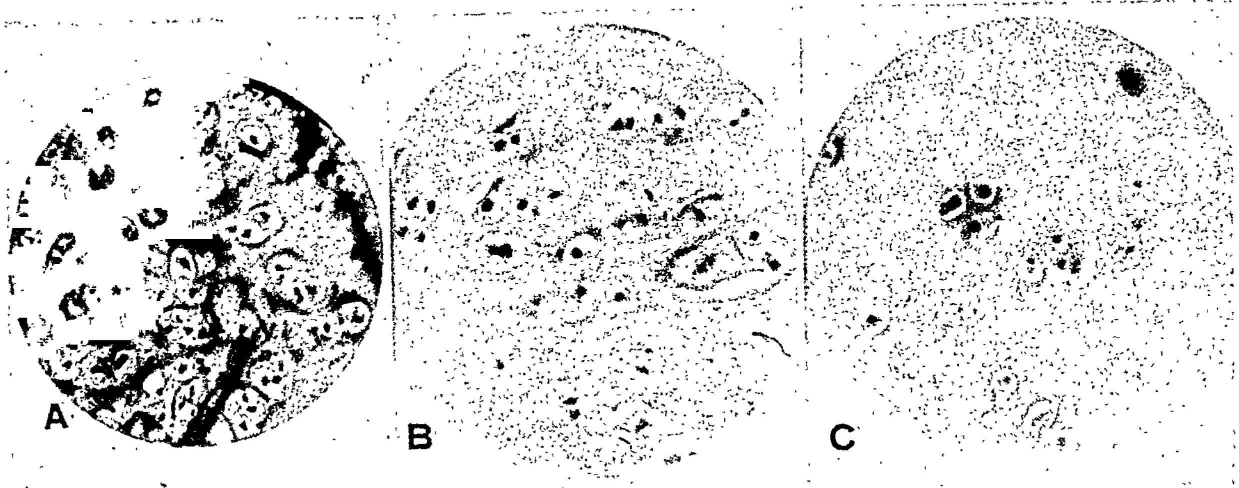


FIG. 24. A. Autogenous human septal cartilage graft buried 1 year, 14 days in abdominal fat. Note normal appearance of cartilage cells compared to those in control section of fresh cartilage, which has been fixed and stained (see fig. 23). ×270.

B. Autogenous human alar cartilage graft buried in abdominal fat for 10 years. Note normal cells. ×270.

C. Autogenous human rib cartilage graft buried in abdominal fat for 20 years. Note normal appearance of chondrocytes. A portion of this graft sectioned in the fresh state contained chondrocytes which completely filled their lacunar spaces and took supravital dyes as living cells. ×270.

a solution of normal saline to prevent drying and death of the cartilage cells. These sections are placed on a slide, covered with a few drops of normal saline and examined under the microscope.

Fresh cartilage cells will be clearly seen as living entities completely filling their lacunae, or occasionally floating in the intercellular fluid which fills the lacunae. The cytoplasm is reticular and contains a number of droplets, possibly lipids or fats. The nuclei are large and well demarcated from the cytoplasm and so faintly stippled that they appear rather homogeneous. *Obviously these are living chondrocytes.*

As the solution of saline evaporates, the cartilage cells will be seen to contract from the cells of the lacunae, so that they resemble the dead cells in fixed sections of cartilage.

One may also apply dilute solutions of supravital dyes to the fresh sections of a cartilage graft and note that only the cytoplasm of the cells takes the dye, thus demonstrating that the cells are viable or living. As the saline solution evaporates, the nuclei of the chondrocytes begin to take the dye, which indicates death of the cells owing to desiccation.

Autogenous rib-cartilage grafts buried for 8, 20, 25 and 27 years were examined in the fresh state and stained with supravital dyes. All of these grafts appeared to contain living cells.

When autogenous septal cartilage grafts buried for 2, 3 and 5 years were also examined in a similar way, all were found to contain living chondrocytes.

Growth of Young Human Cartilage Grafts

Dupertuis in 1941 reported definite evidence of growth in young rabbit cartilage (fresh autogenous and homogenous) grafts from the ears and ribs. In 1946 I noted a small increase in some young human rib-, septal- and ear-cartilage grafts buried up to 2½ years, whereas others did not show an increase in size.

Later experimental work by the author with young rib-cartilage grafts buried 6, 7, 7½, and 8 years *did not demonstrate* evidence

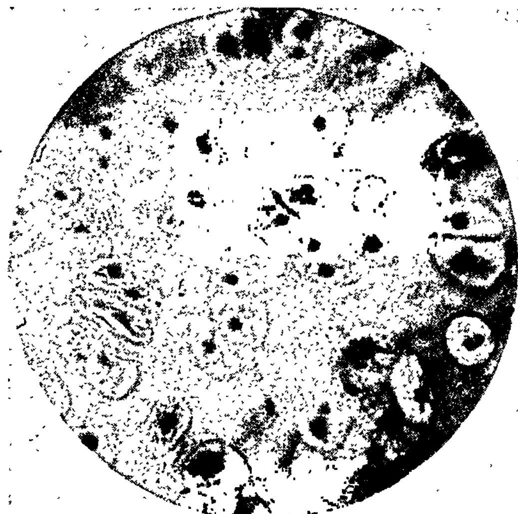


FIG. 23. Control fixed and stained section of human costal cartilage. Note the general appearance of the cartilage cells, which have contracted somewhat from the walls of the lacunae due to fixation. These are the more centrally located chondrocytes. Chondrocytes near the external surface of cartilage are more spindle-shaped and these do not tend to retract from the walls of their lacunae after fixation and staining. If thin shavings of fresh cartilage are immersed in normal saline and examined under the microscope one notes that all of the chondrocytes completely fill their respective lacunar compartments. These are cells observed in the living state. When the saline solution evaporates the cells retract from the walls of their lacunae and resemble the cells in fixed sections of living cartilage. ×430.

the child is older before completing final work on the reconstruction.

Autogenous rib-cartilage grafts are like all other autogenous grafts in that they do not always behave in a given manner following transplantation. Free skin grafts, which usually contract only moderately following transfer, will occasionally be reduced to one tenth of their original surface area, particularly in children; bone grafts in contact with bone react variously. And who can predict the behavior of a free fat graft?

Autogenous rib-cartilage grafts in general, however, tend to retain their same general bulk following transplantation. The chondrocytes survive as living cells, which maintain the cartilaginous character of the matrix. Although bone formation may occur either in or outside cartilage grafts, one does not see any *new cartilage formation* in young or adult human grafts, such as had been reported in animal cartilage grafts.

Host Tissue Reaction

Examination of autogenous rib-cartilage grafts shows the presence of a dense connective-tissue capsule surrounding grafts that have been buried in human abdominal fat for two weeks. A very moderate cellular reaction is present in the host fatty tissue just outside the connective-tissue capsule. This consists of a general infiltration by polymorphonuclear leukocytes and large and small mononuclear cells, the small ones being identified as lymphocytes. There are also a proliferation of new young fibroblasts, engorgement of blood vessels and some new blood vessel formation. Occasional eosinophiles and plasma cells have been seen. The cellular reaction in the host tissues about autogenous grafts subsides rather rapidly, so that after one month it is not very active and often cannot be identified two months following transplantation. The thick connective-tissue capsule thins out somewhat with time, and appears to serve as a substitute perichondrium in which the cartilage is both well nourished and comfortable.

Examination of Fresh Section of Autogenous Rib and Septal Cartilage Grafts

Additional evidence that the cells in autogenous rib and septal-cartilage grafts survive as living chondrocytes has been demonstrated in the following way.

Fresh cartilage grafts, soon after removal, are placed in melted paraffin which is about to solidify, and the paraffin containing the graft is immersed in ice water to hasten solidification and prevent heat damage to the cells.

The paraffin block is then placed in a microtome and the thin shavings caught in

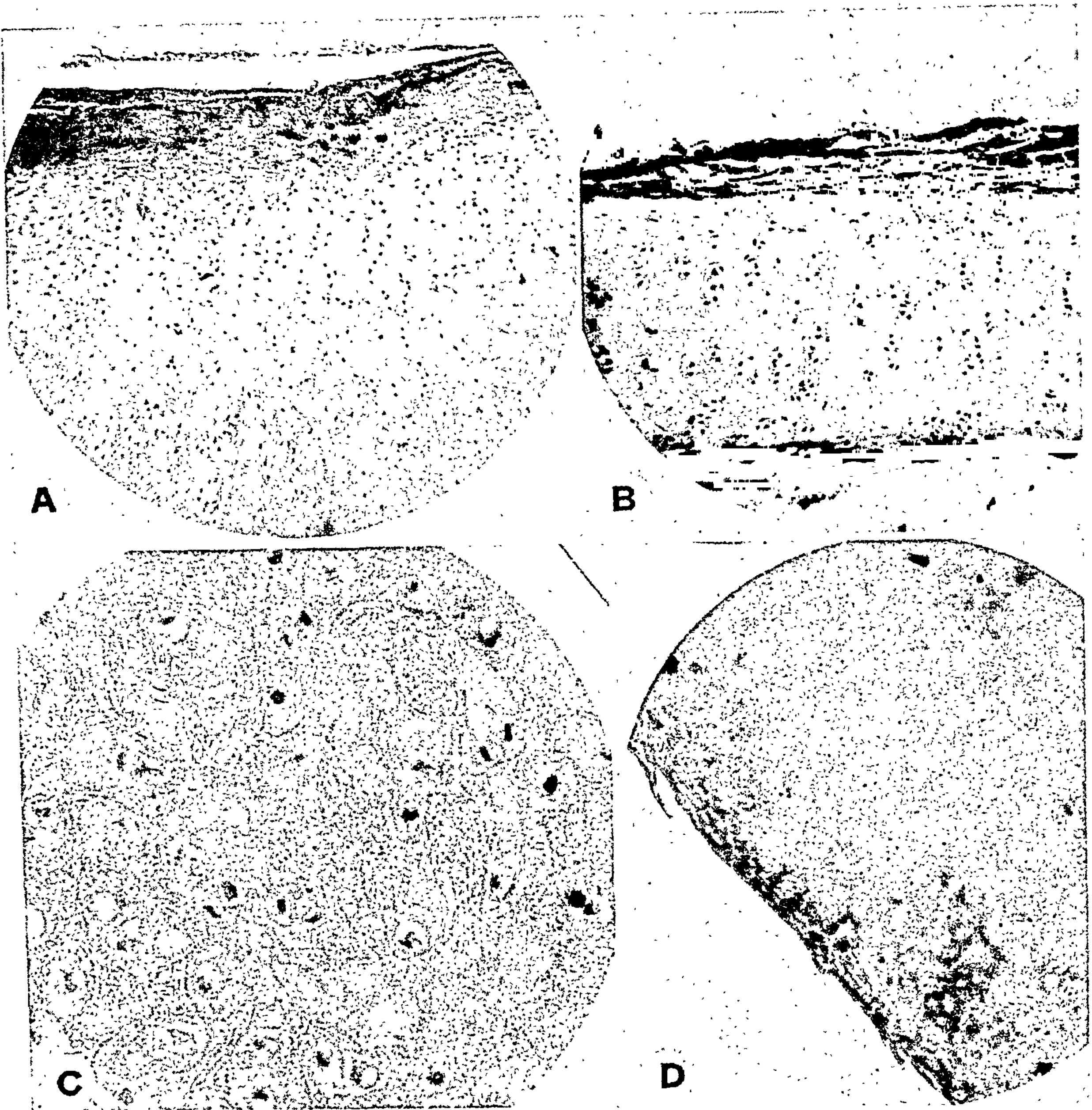


FIG. 26. A. Autogenous adult human septal cartilage graft buried without perichondrium for 5 years. Note absence of invasion and absorption. In a section of this graft in the fresh state the cells appeared as normal living cartilage cells. Thirty-two additional autogenous septal cartilage grafts buried for shorter periods also gave no evidence of invasion or absorption.

B. Autogenous adult human alar cartilage graft buried 4 ½ years. Note absence of invasion and absorption. In fresh sections of this graft the cells appeared as living cartilage cells. Seven additional autogenous alar cartilage grafts also gave no evidence of invasion or absorption.

C. Autogenous human elastic ear cartilage graft buried for 4 years. When a section was made of this graft in the fresh state the cells appeared as living cartilage cells. Twelve additional ear cartilage grafts buried for shorter periods also showed no evidence of invasion or absorption.

D. Autogenous human rib cartilage graft buried without perichondrium for 14 years. Note absence of invasion and absorption. In fresh sections of this graft the cells appeared as normal living cartilage cells. Fourteen additional autogenous rib cartilage grafts also showed no evidence of invarion or absorption. From L. A. Peer, Plast. & Reconstr. Surg. 1: 3, 1946.

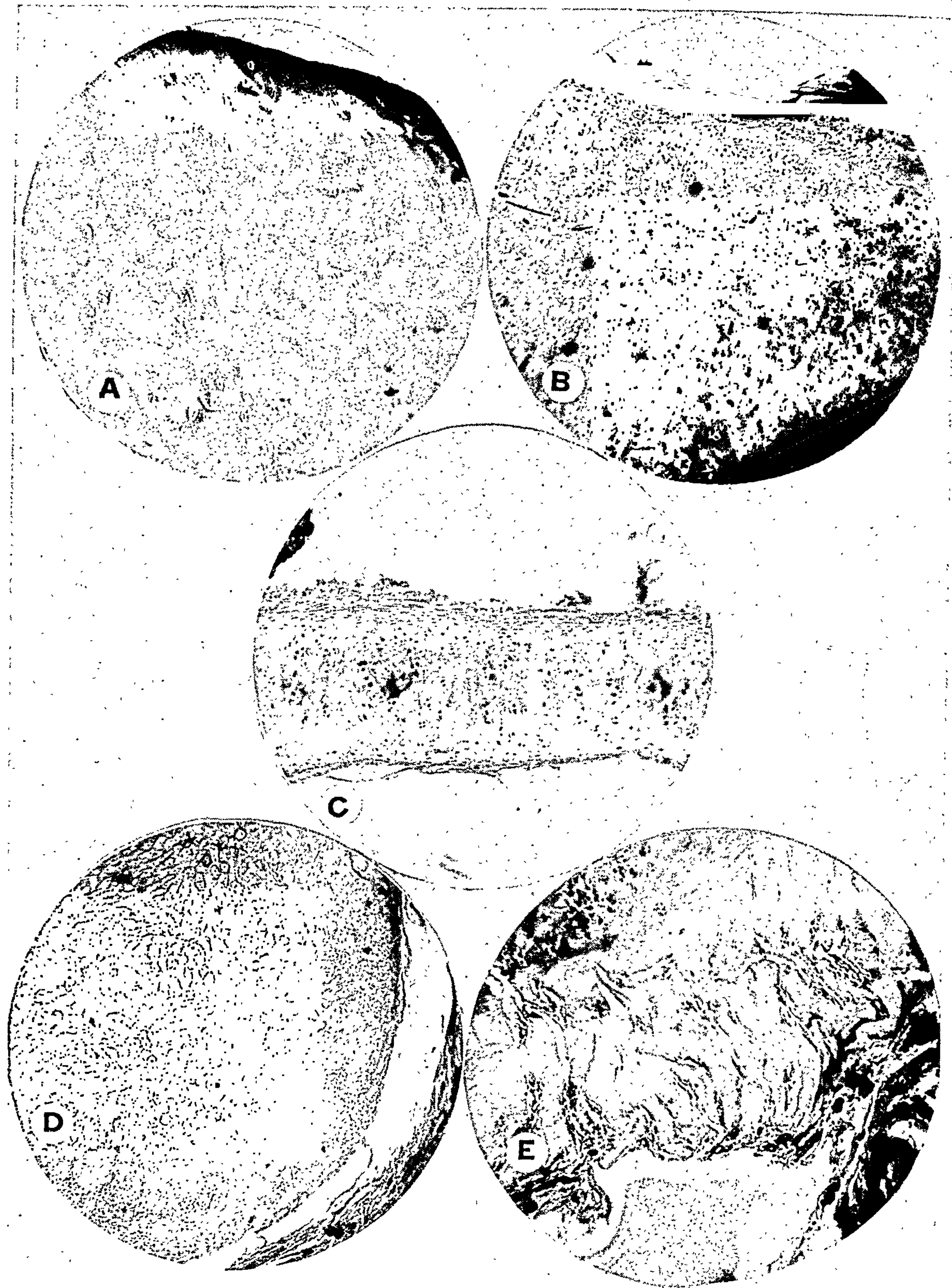


FIG. 25. Various types of cartilage grafts.

A. Low power magnification of an autogenous rib cartilage graft buried 12 years. Note complete absence of invasion or absorption.

B. Autogenous septal cartilage graft buried 3 years. Note absence of invasion and absorption.

C. Autogenous alar cartilage graft buried 2½ years. There is no evidence of invasion or absorption.

D. Autogenous ear cartilage graft buried 3 years. Note absence of invasion or absorption. Under high power magnification the cartilage cells in all of these autogenous grafts appear like living cells.

E. Preserved cadaver rib cartilage graft buried 18 months. Note invasion of cartilage by strands of connective tissue and loss of cartilage cell structure.

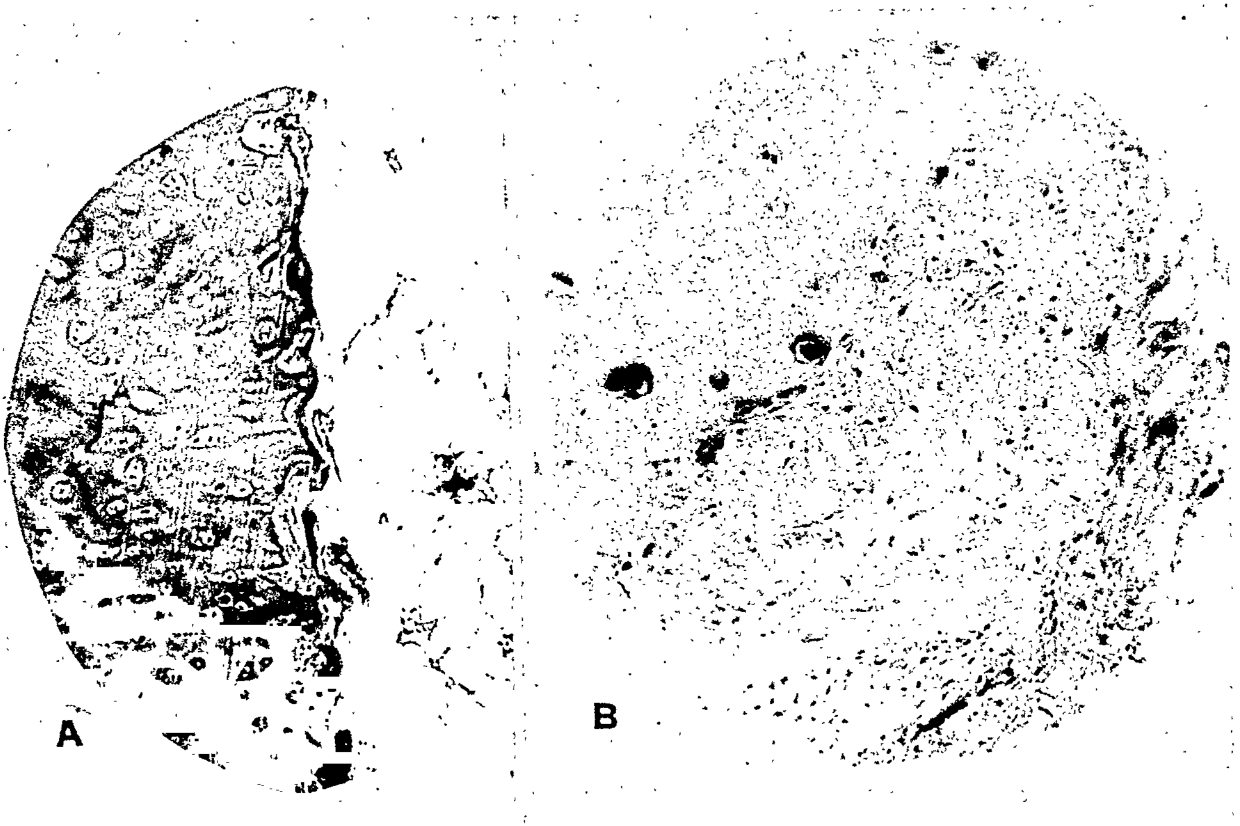


FIG. 27. A. Autogenous human rib cartilage graft buried in the nose for 6 years. Note slight penetration of host tissue into the outer surface of the graft. These small indentations probably represent nicks made in the cartilage at the time of transplantation which were later occupied by host fibrous tissue. ×100.

B. Autogenous human rib cartilage graft buried in the nasal tissues for 20 years. In this graft there is definite invasion and absorption at the outer margin of the transplant. The bulk of the cartilage graft is retained, however, and the cartilage cells are viable. ×430.

homogenous rib-cartilage graft buried for 3 years and 9 months in human abdominal fat showed no definite evidence of invasion or absorption. The chondrocytes in this fixed and stained section appeared as living cells when compared with those in fixed and stained control cartilage.

Microscopic examination of other fresh homogenous rib-cartilage grafts, buried for 1, 2, 2½, and 3 years, all showed definite invasion and partial absorption of the matrix. Re-examination of these fixed and stained sections by the author recently demonstrated that the unabsorbed matrix was quite normal in appearance,[1] and that the cells appeared like the chondrocytes in control cartilage sections. Certainly the cells were not the small bits of granular debris found in preserved cartilage grafts and, moreover, a number of the centrally-located cells and all of the spindle-shaped cells near the periphery were quite normal in appearance; apparently the chondrocytes encased in their matrix armor were living cells. Where the matrix had been removed by invading host tissue, there was a complete absence of cartilage cells.

The author recently removed a fresh rib cartilage homograft which had been buried in abdominal fat for 4 years. This graft, without perichondrium, was removed from a 3-year-old donor and transplanted into a 4-year-old unrelated patient. The graft when

[1] So-called "fibrillation" was present in some areas of the matrix. This is due to a breakdown in the binding substance which allows the collagenous fibers to be visualized.

of growth. These grafts were buried without perichondrium and with perichondrium attached, in abdominal fat.

Reports have appeared in the literature which suggest the growth of autogenous rib-cartilage grafts inserted in the nose but such determinations, being obtained clinically by palpation and by measurement of a graft covered by nasal skin, are not completely reliable. There is, of course, the possibility that the nasal tissues are an especially favorable transplantation site.

The available evidence at this time indicates that young human cartilage autografts *do not increase in size following transfer;* at any rate they do not increase in size consistently, and the growth principle cannot be depended upon in clinical procedures on children. Young human cartilage homografts are slowly but progressively reduced in size. Autogenous cartilage grafts inserted in the columella or in the dorsum of the nose in growing children appear to influence the growth of the alar and lateral cartilages and the covering skin, which are established structures with intact circulatory systems. The evident growth of these tissues, due to the mechanical support of the free cartilage transplants, may have been erroneously interpreted as actual growth of the cartilage transplant itself.

Dead Autogenous Cartilage Grafts

Fresh autogenous rib-cartilage grafts often have a tendency to bend and become distorted after transfer. This is apt to occur when the surgeon inserts his graft rather hastily instead of testing it by bending it in different directions and noting the direction of curvature; this distortion can be corrected by partial cuts on the concave surface and occasional V-excision on the convex surface. Sometimes distortion will occur despite these precautionary measures. Gordon New demonstrated that the bending factor in hyaline matrix could be largely removed by heat treatment, such as exposing the fresh rib cartilage to steam or by boiling. The heat treatment, of course, kills the chondrocytes; but that these dead autogenous grafts, from a clinical standpoint, retained their general bulk following transplantation was reported by New.

In our series, sections of heat-treated autogenous rib cartilage buried in abdominal fat for 2, 2½, and 4 years showed evidence of gradual invasion by host connective tissue. (1). The graft buried for 2½ years had been largely replaced by connective tissue, but the other grafts retained much of their original size. One heat-treated autogenous septal cartilage graft was completely replaced by fibrous tissue in one year after transplantation.

The heat treatment of living autogenous cartilage destroys all of the living chondrocytes. One has an aversion to such destruction of the living element in the tissue since, like all other tissues, cartilage survives better as an autogenous graft when the cells remain viable. (Free muscle grafts are the one exception.) The chondrocytes are not just small bodies which happen to be present; they are the parenchymal cells of cartilage and their survival after transplantation is essential to the maintenance of the intercellular matrix.

Fresh Homogenous Cartilage Grafts in Humans

Although many clinical opinions regarding the survival or absorption of fresh homogenous cartilage grafts are found in the literature, there are very few instances in which the grafts have been removed and studied microscopically. The cases where removal and examination of fixed and stained sections were described are mostly single case reports, rather than controlled experimental burial and removal at selected intervals.

In our experiments, sections of a fresh

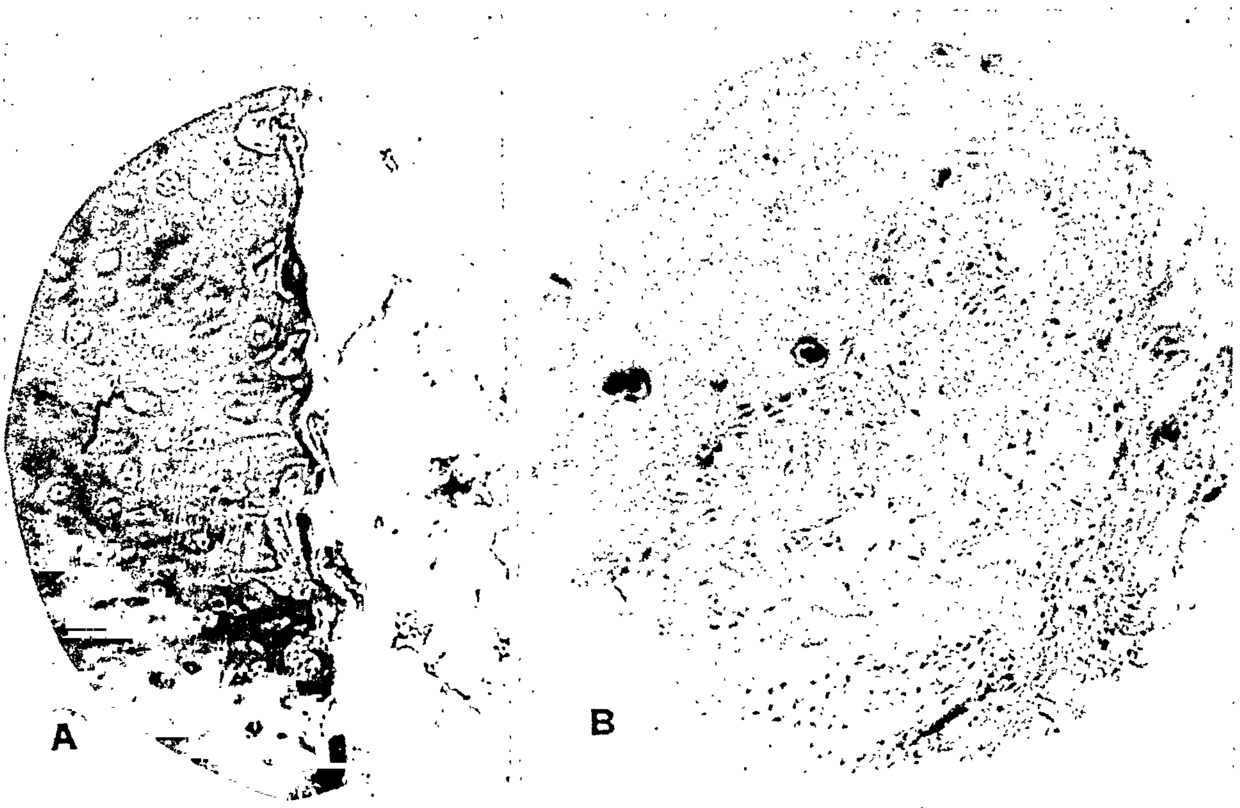


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[1] So-called "fibrillation" was present in some areas of the matrix. This is due to a breakdown in the binding substance which allows the collagenous fibers to be visualized.

acceptance of similar ants from another colony.

The fact that the cells in fresh cartilage homografts survive so long has possibly been explained by the mucoprotein theory of Bacsich and Riddell.

Significance of the Mucoprotein Content in Matrix

Bacsich and Riddell (2) have contributed a very interesting hypothesis to explain the long survival time of cartilaginous, lens and corneal homografts which they noted in animal experiments. The capacity of the cells in fresh cartilaginous, lens and corneal cross-grafts to survive is owing to similar mucoproteins in the ground substance which serve as a protective armor against host antibodies. These investigators point out that this mucoprotein substance resembles chemical substances found in the capsules of pneumococci, which have the ability to form and increase the thickness of this capsule for protection against hostile antibodies in the blood.

The protective capacity of mucoprotein in the cartilaginous matrix was certainly demonstrated by examination of the fresh human homograft buried for 4 years (in the author's experiments). In this transplant chondrocytes remained viable when they were surrounded by only a thin wall of matrix separating them from ingrowing host capillaries and fibroblasts. In some areas the matrix measured about half the diameter of the chondrocyte; but the latter was still living, as demonstrated by microscopic examination and characteristic supravital staining reaction.

Bacsich and Wyburn (3) accredit Borst (4) as the first to present the basic conception that each individual should be regarded as a specific biochemical system, and within this common background the different organs and tissues work together but preserve their own characteristics.

On such a foundation, strengthened by the results of his own extensive experiments, Loeb (5) built his conception of the biological basis of individuality. According to his thesis, the individuality of a tissue is a summation and integration of qualities in respect to its identity as a particular tissue or organ, in respect to the organism of which it is a part, and in respect to the species and order of this organism (genetic relationship). The autograft is therefore attuned to the biochemical system of which it is a part, and is accepted as a transplant. The host reacts to the homograft (and more so to the heterograft) and makes an effort to destroy it. Loeb noted the capacity of cartilage and cornea to survive long after homografts of other tissues and organs had been destroyed; this he regarded as evidence of low tissue specificity but he did not determine the

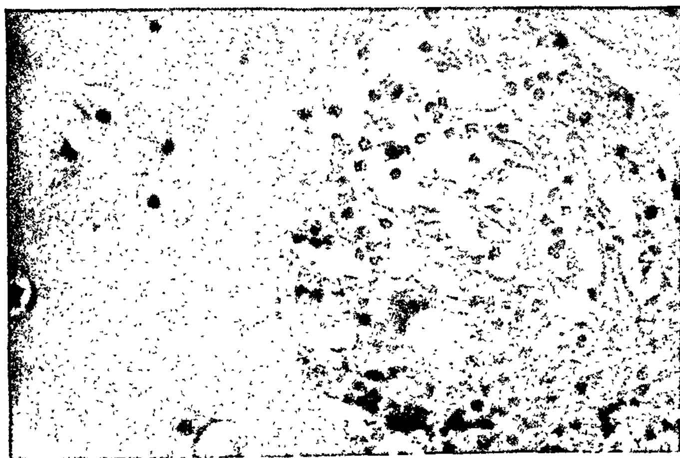


Fig. 30. Fresh homogenous human cartilage graft buried 4 years, under high power magnification. Note area where host cells have invaded the cartilage. The cartilage cells protected by their matrix armor appear as living cells. Where the matrix has been removed the cartilage cells disappear among the large numbers of proliferating host fibroblasts. Dead or dying chondrocytes were never seen. The main host cellular agency seen in areas where cartilage is being absorbed is the fibroblast, which is always accompanied by host capillaries. ×390.

reason why these particular tissues are less readily overwhelmed by those of the host.

Bacsich and Riddell have suggested the protective properties of the mucoprotein content of cartilage, lens and cornea as the real reason for the survival of these tissues, and this theory is impressive since it fits harmoniously with numerous isolated observations regarding the behavior of various homografts.

Wyburn (6) believes that whether one accepts the mosaic conception of organismal differentials conceived by Loeb, or is content with the more general interpretation of the host treatment of homografts as a serological defense against invasion by living foreign elements (7, 8), there is no doubt whatever about the measure of protection afforded to tissues rich in mucoprotein. It may be that this protection is but a function of the viscous nature of the mucoprotein which prevents the diffusion from the graft tissues of those substances which provoke aggressive host tissue reactions against the graft. It is also possible that the mucoproteins play a more active rôle.

Many unsuccessful attempts have been made to prolong the life of homografts other than those of cartilage and cornea—usually by some form of treatment of the graft before insertion. Some degree of success might be achieved if it were possible to endow the grafted tissue with those qualities bestowed on cartilage, lens and cornea through their mucoprotein content (Bacsich and Wyburn).

Chondrotin-sulfuric acid in the matrix of hyaline cartilage and *mucoitin-sulfuric acid* in the intercellular substance of the cornea are chemical isomers. Chemical analysis, staining and enzymotic reactions all indicate that the various mucoproteins of cartilage and cornea have much in common, and this fact is reflected in the common qualities of *transparency* and *avascularity* (9, 2).

To recapitulate, fresh cartilage homografts in man often retain their cartilaginous structure for long periods of time; in other instances the graft structure is rather rapidly invaded and replaced by host fibrous tissue. In general, the tendency to invasion and absorption is a progressive one.

The chondrocytes in fresh homografts survive as living cells and continue to be viable as long as they are protected from invading host tissue and host antibodies by even a thin armor of matrix. The host cells, which in the main are young fibroblasts accompanied by blood vessels, appear to absorb the matrix structure slowly. Where this has been completely removed no chondrocytes are visualized; presumably they are destroyed by substances in the host tissue fluid, *but the author has never actually seen a dead or degenerating dehyalinized chondrocyte.*

The human chondrocyte in a fresh homo-

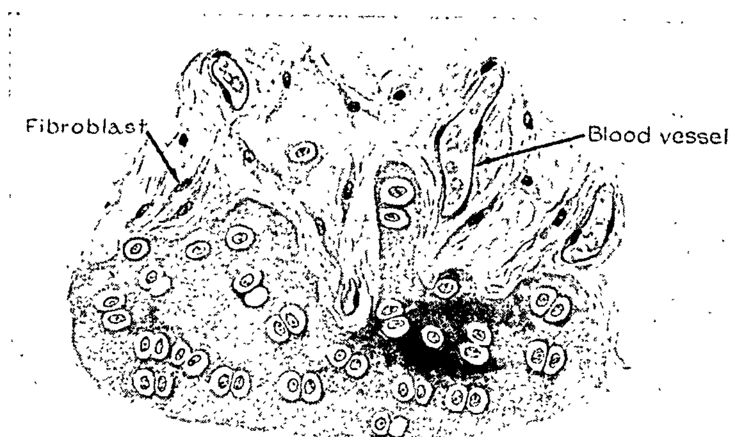


FIG. 31. Fresh homogenous human cartilage graft buried 4 years and sectioned in the unfixed state (drawing of slide). Host fibroblasts accompanied by blood vessels have penetrated and absorbed large portions of the homograft. The chondrocytes in the graft, however, appear as living cells as long as they are surrounded by a protective layer of hyaline matrix. When the matrix is removed the cells disappear among the large numbers of invading fibroblasts. Dead or dying cartilage cells were not observed in this or any other fresh homogenous cartilage graft.

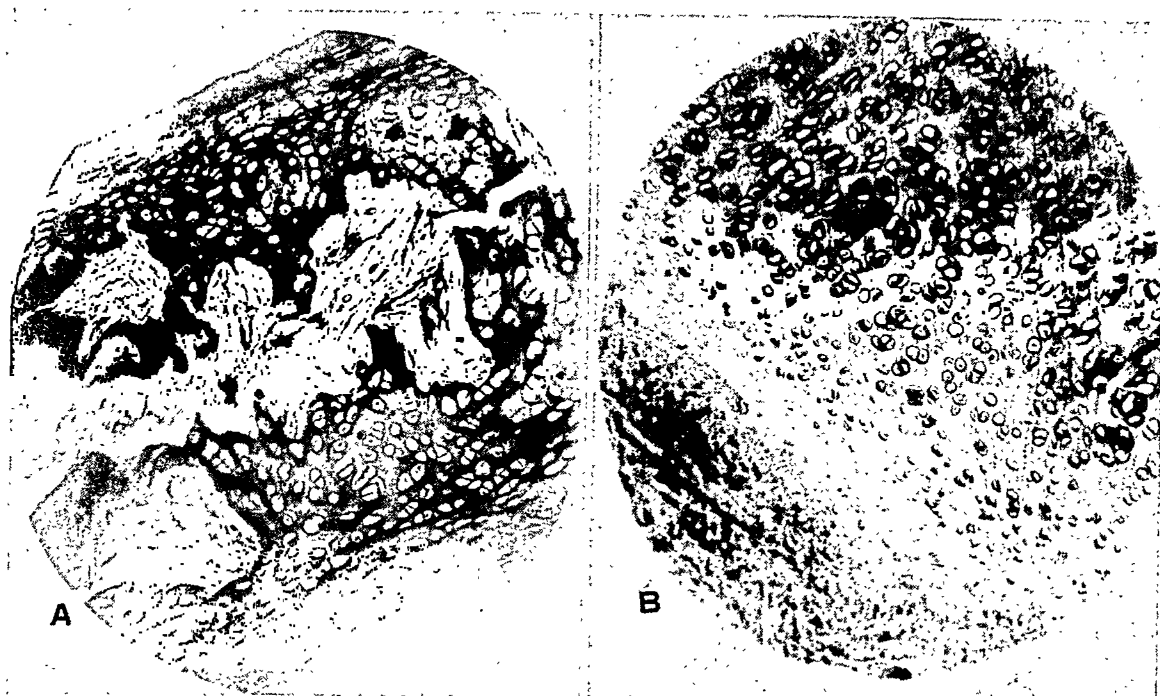


FIG. 32. A. Preserved cadaver cartilage graft buried 18 months. Note beginning invasion and absorption of graft.

B. Living autogenous rib cartilage graft buried 25 years. Note normal appearance of graft and absence of absorption.

graft does not have the ability to retain its matrix or to produce new matrix as the host tissues remove this hyaline substance. In animals new cartilage formation in fresh homografts has been noted by investigators, but in humans the cells in fresh cartilage homografts lack this ability regardless of the age of the donor and that of the recipient. It would be interesting to transplant fresh cartilage heterografts from young rabbits into humans in order to observe whether or not the chondrocytes survive, and also their capacity for new cartilage formation.

The growth property demonstrated by Dupertuis in young rabbit cartilage homografts appears to be entirely absent in young human homogenous cartilage which, to the contrary, decreases in size due to absorption.

In general, fresh cartilage homografts in man are gradually absorbed and reduced in size to the same extent as preserved homografts. Preserved homografts in a cartilage bank, being readily available, are therefore used much more frequently than fresh homografts.

Preserved Cartilage Homografts

Preserved rib-cartilage homografts, introduced by Pierce and O'Connor, are a valuable contribution to plastic surgery and may be regarded as the material of second choice when it is not feasible to use the patient's own cartilage. This opinion is based on wide clinical use of preserved rib-cartilage grafts by a large number of plastic surgeons over a period of twenty years. The metals, vitallium and tantalum, and other substances such as polyethylene and various resins are constantly being buried by one group of surgeons and constantly being removed by another group of surgeons after varying periods of time, depending upon individual tolerance of the patient. Preserved rib-cartilage grafts do show a tendency to gradual invasion and absorption, but they are at least well tolerated in human tissues and

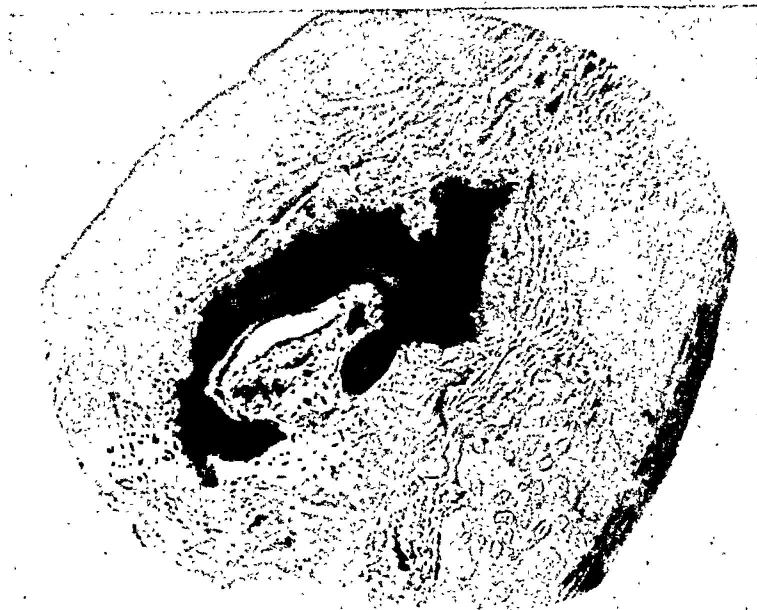


Fig. 33. Homogenous human septal cartilage graft stored in alcohol and buried in abdominal fat. When this graft was removed 14 months after transplantation an area of true bone formation was seen in the center of the graft. Note long chain of osteoblasts and occasional osteoclast. The bone is supplied by ingrowing host blood vessels, as is always true wherever bone is found in the human body.

seldom give rise to late inflammatory reactions resulting in extrusion of the grafts.[2]

Most hospitals with plastic services have a preserved cartilage bank, as described by Barrett Brown (10). Cartilage obtained at fresh autopsies is refrigerated (frozen if possible), all soft tissue including the perichondrium removed, and again refrigerated in a solution of aqueous merthiolate.

The author usually preserves cartilage in 70 per cent alcohol and stores it at refrigerator temperature. Grafts appear to retain their bulk equally well when preserved by either of these methods.

Histologically, preserved rib-cartilage grafts are well tolerated by the host tissue. The initial reaction is more severe than the cellular activity occurring in host tissues surrounding autogenous grafts, and it tends to remain for a longer time. A larger number of lymphocytes, plasma cells, eosinophiles and polymorphonuclears are noted in the host tissue surrounding the foreign grafts and occasional giant cells will usually be seen on careful examination. A thick connective-tissue capsule is formed around the preserved cartilage graft and only very rarely does the graft incite inflammatory reaction resulting in extrusion.

In a significant number of preserved rib-cartilage grafts the host fibrous tissue slowly but progressively invades and absorbs the graft structure, so that the graft becomes reduced in size and completely absorbed as an ultimate effect. True bone formation often occurs in preserved cartilage grafts and this is helpful clinically, since the new bone substitutes for the cartilage matrix which has been removed. This new bone is in the nature of a free bone graft in soft tissue; at any rate it is not in contact with bone and its ultimate fate is not known. The new bone in cartilage grafts appears to be endochondral, since dead cartilage is replaced by bone. The source and origin of the osteoblasts, osteoclasts and bone cells clearly seen in these sections are somewhat speculative. The question arises—where do they come from? Many of our grafts were transplanted into abdominal fat and not in contact with bone, periosteum or even fascia. Can undifferentiated connective-tissue cells present in the fat take on specialized function, or do undifferentiated cells with this capacity migrate to the area?

Occasionally preserved rib-cartilage grafts

[2] The author is indebted to Warren Davis, Staige Davis, Claire Straith, DeHoyte Klein and others for sending specimens of preserved rib cartilage grafts for microscopic examination.

are only moderately invaded by host tissue (as seen in sections buried for 12 years) but this is an exception to the general rule. Occasionally, also, a preserved rib-cartilage graft will be invaded and absorbed rather rapidly.

Preserved septal, alar, lateral, auricular, and meniscus cartilage homografts are also well tolerated by human tissues. Sections of preserved septal cartilage grafts removed and examined histologically (1) show about the same changes as preserved rib cartilage grafts. There is a clinical impression, however, that these grafts undergo more absorption—but this is possibly due to the fact that they are used to fill very slight depressions, where a small degree of absorption would be quite apparent. Preserved meniscus, which is fibrocartilage, appears to be resistant to absorption. Whether it is superior to preserved rib cartilage is debatable until a larger number of grafts buried for longer periods of time have been examined. It is hoped that Vidaurre, Mir y Mir, and Backus will continue to use this fibrocartilage graft so that later definite reports will be available. Preserved hyaline, elastic and fibrocartilage grafts all tend to retain their specific matrix structure following transplantation unless this matrix is absorbed and replaced by fibrous tissue or bone. Living cells from the host tissue do not occupy the empty lacunae and become cartilage cells, and new cartilage formation has not been observed in or about dead preserved cartilage grafts of any variety.

Transplantation of Epiphyseal Cartilage

There is disagreement among investigators regarding the success of epiphyseal cartilage transplants. The available evidence is that it does not maintain its special function of producing growth in the length of a bone after transplantation.

When the epiphyseal cartilage is transplanted together with a part of its bony diaphysis, and the diaphysis is in contact with host bone, the transferred diaphysis may heal by bony union and the joint may function. There is disagreement, however, regarding the long-term survival of the epiphyseal cartilage as such.

Both autogenous and homogenous joint transplants have been reported in the human with varying degrees of success, and the procedure still appears to be in the experimental stage of development.

Ollier (11) in 1867 and Tizzoni at a later time both noted that articular cartilage degenerated when transplanted subcutaneously into animals. Both of these early investigators concluded that the presence of synovial fluid was essential for the survival of epiphyseal cartilage, and this belief still persists today.

In animal experiments concerned with the transplantation of epiphyseal cartilage, Helferich (12), Rehn and Wakabayashi (13), von Tappeiner (14), Obata (15) and Heller (16) all report some degree of subsequent longitudinal growth of bone.

Heller in particular reported good results in 120 animal experiments and he concluded that autogenous reimplantation of the epiphyseal plate was successful and that the procedure offered a promise of favorable application in man.

Fohl (17), also transplanting epiphyseal cartilage attached to its bony diaphysis into animals, believed that the successful results justified clinical application in man.

Contrariwise, Haas (18) has opposed the procedure because he believed that the epiphyseal cartilage plate loses its power of causing growth of bone after transplantation and that a surgeon is not justified in attempting such a transplantation in a patient with the expectation of obtaining longitudinal growth of bone. Bisgard (19) also was opposed to the procedure because of the uncertain result.

Lexer (20) made the first clinical applica-

tion of whole-joint transplantation in man with homogenous bone and cartilage. In his best-known case he used a resected knee-joint homograft from a freshly-amputated limb and the case was reported 6 years after operation. The patient had good weight-bearing ability and sufficient motion at the joint for locomotion. Recent roentgenographic examination showed absorptive changes at the point of union of the bone graft with host bone. The condition of the epiphyseal cartilage was not determined. Lexer made other attempts to transplant knee joints but these were not successful.

According to Wenger (21), the only successful autogenous transplantation performed on a human being from the viewpoint of growth was reported by Straub in 1912. Straub (22) transplanted a piece of diaphysis with the epiphyseal line and part of the epiphysis in a child 6½ years old. A follow-up record made 17 years later (1929) demonstrated that the epiphysis had survived, grown and continued to function physiologically. Haas challenged this statement, however, and held that no longitudinal growth of bone had taken place.

Wenger in 1945 reported an interesting case in which he transplanted into a child of 7 years the epiphysis of the fibula and part of the attached diaphysis (autogenous graft) to the first metatarsal bone, which had been almost completely destroyed. The toe, which was greatly shortened prior to operation, was immediately corrected by the transplant. Observations made over three years demonstrated that the transplant had "taken" and was functioning physiologically.

In experinents on dogs Herndon and Chase (23) drew the following conclusions. Some of the bone cells in autogenous whole joints remain viable following transplantation. The bone is rapidly revascularized and the dead bone is replaced by new bone. The deeper layers of cartilage cells remain viable, but some degeneration of the articular surface usually results. All of the cells in delayed homogenous transplants and most of the cells in direct homogenous whole-joint transplants die. Revascularization and active osteogenesis are slow. The grafts unite and function satisfactorily for six to eight months, but degenerative changes similar to those seen in aseptic necrosis begin between four and six months after transplantation. These changes may progress to complete disintegration of the joint. Since similar changes occur in direct homogenous transplants as in delayed homogenous transplants, the method of preservation apparently is not the important factor in the early degenerative changes. The factor that produces these degenerative changes, which are so much greater than those in autogenous whole joint transplants, has not been determined. On the basis of experimental data, Herndon and Chase believe that satisfactory results might be obtained in humans with autogenous transplants, particularly in non-weight-bearing joints such as the small joints of the hand.

In a case reported by Capurro and Pedmonte (24), a fresh homogenous femur from a girl who had just died from an accident was inserted in place of an entire femur removed because of hydatid cyst. The fate of the cartilage after transplantation was not accurately determined.

Later sporadic case reports on homogenous and autogenous joint transplantation in humans have not added to our knowledge regarding this interesting subject.

The author buried the bone and joint structures of a complete supernumerary toe and finger respectively in the abdominal fat of the same infant. There were two bones in the toe and three bones in the finger, and all joints were enclosed by the capsule coverings. The nails were left on the terminal toe and finger bones and the periosteum was also left attached to the bones. In roentgenographic examination and on palpation 22 months after transplantation, the bones

and cartilage were found to have retained their structure. Surprisingly, the bones can be readily flexed and extended by palpation through the skin, indicating that the joint surfaces and capsules have survived and that the separate bones have not become joined together or absorbed and replaced by fibrous tissue.

Summary Comment

It appears that the issues at stake in autogenous and homogenous joint transplantation involve the successful transplantation of autogenous or homogenous bone in contact with bone at one end, the survival of the autogenous or homogenous epiphyseal cartilage attached to the free end of the bone, and retention of the growth capacity in the cartilage and in the bone at the epiphyseal line. Functional considerations such as stability and weight-bearing as well as increment are an important factor in the leg bone, whereas only stability and increment are necessary in the non-weight-bearing bones.

There is evidence in autogenous joint transplants that the bone graft heals and either survives or is replaced by the host bone. There is evidence also in homogenous joint transplantation that the bone may make bony union with the host bone and possibly be replaced in kind by creeping substitution. The fate of the epiphyseal cartilage in autogenous and homogenous transplants and the capacity of the cartilage to grow are surrounded with considerable doubt. Since autogenous tissues are always the material of choice for grafting purposes (and this applies even to corneal grafts) it seems probable that the possibilities of success are greater when autogenous joints are utilized. Young costal and septal cartilage grafts do not appear to grow in the human and the growth capacities of young human epiphyseal cartilage have not been clearly demonstrated.

Other autogenous cartilage grafts such as costal, septal and ear cartilages survive transplantation as living tissues and it seems probable that autogenous epiphyseal cartilage may also survive. Fresh homogenous costal cartilage grafts also survive in the human but there is slow, progressive absorption of the matrix of these grafts by host tissue (young fibroblasts) and the chondrocytes in the graft disappear as their protective matrix is removed.

Thus it appears, in whole or half joint transplantation, that autogenous transplants are preferable to homogenous transplants whenever the former are available.

One is inclined to doubt the statement that epiphyseal cartilage is nourished entirely by the synovial fluid in the joint cavity rather than from the adjacent bone.

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CHAPTER 13

Transplantation of Cartilage in Humans
(Continued)

HETEROGENOUS CARTILAGE GRAFTS IN HUMANS

A wave of enthusiasm for free grafting affected the medical profession following the pioneer work of Ollier and Thiersch with free skin grafts, and little distinction was drawn regarding the source of the grafts, whether they came from the patient's own tissues, from the tissues of another human or from animals. Bone and cartilage heterografts fixed in formalin or some other preservative were quite popular, because these hard resistant structures could be buried in human tissues and their persistence noted by palpation for relatively long periods of time following successful transfer.

Recorded Ox-Cartilage Grafts

Stout (1) in 1933 decided to give ox-cartilage heterografts a fair trial before condemning their use in humans as a grafting material. He used formalin-fixed ox cartilage, which was placed in sterile water to remove the formalin before transfer. From a clinical standpoint these dead zoografts gave satisfactory evidence of persistence as rigid supporting structures for saddle nose deformities.

The possibility of bovine cartilage as a grafting material was also investigated by Wardill and Swinney (2) in 1947, the xiphisternum proving to be the most suitable for surgical purposes. This cartilage, after being removed in an abattoir, was placed in dry bottles; the soft tissues and perichondrium were dissected off. The cartilage was then parboiled (in boiling water for one minute) and preserved in merthiosaline. The preserving solution was renewed the next day and once a week thereafter. In a case of saddle nose a graft of bovine cartilage was introduced into the tissues through a mid-line columellar incision. The result of the graft was excellent. The patient has a normal-looking nose. The results in all the patients treated in the same way have been equally satisfactory. The grafts seem to resist infection better than fresh autogenous cartilage, and the surrounding tissues show practically no reaction whatsoever.

Although Stout (1933) first described the use of ox-cartilage grafts in plastic surgery, it was not until Wardill and Swinney (1947) reported their results that a serious interest in the possible value of this material was stimulated.

Gillies and Kristensen (3) used ox-cartilage grafts preserved in merthiolate solution (1:4000) on 125 patients for correction of

nasal, ear and facial deformities. The percentage of successes, from a clinical standpoint, was estimated at 95 per cent in nasal grafts, 50 per cent in ear grafts and 80 per cent in restoring other facial contours. On one occasion Gillies and Kristensen had an opportunity to examine a successful eight-month-old ox-cartilage graft transplanted in the cheek. Grossly, the graft was surrounded by the usual connective-tissue capsule and beneath this the graft appeared like normal cartilage. Microscopic examination demonstrated that the cells with dark-staining nucleoli were almost as nicely preserved as in control sections of preserved ox cartilage. The matrix of the graft appeared quite normal, with no degenerative changes. A number of plasma cells, some leukocytes and two giant cells were observed to be present in the host tissue surrounding the graft.

This interesting microscopic examination of preserved ox cartilage buried in human tissues is the first report on the subject in the literature. The cells in the graft, of course, were dead chondrocytes, and the fact that they had dark-staining nucleoli and so forth is simply a tribute to the preservative. The absence of invasion or absorption of the matrix and its normal appearance at eight months following transplantation are impressive; but the presence of scattered plasma cells, leukocytes and giant cells in the host tissues surrounding the graft even after eight months indicates that the host is aware that it is harboring a foreign tissue graft and is planning for the eventual absorption.

In a comprehensive article Gibson and Davis (4) reported that in 12 cases of bovine cartilage implants in man, ten had been absorbed within two years. They also transplanted a series of bovine cartilage implants into human volunteers. In these instances each successive implant showed a degree of absorption greater than the preceding, thus indicating the progressive development of a systemic response. Absorption occurs first by surface erosion, then by fibrous replacement and later by dissolution of the cartilage as the host's reaction increases. The rate of destruction of the cartilage is related to the surface area of the implant. Gibson and Davis found that a single thick portion of cartilage such as is used for nasal repair will survive for a much longer period than will a corresponding mass of diced bovine cartilage which exposes to the host's tissues a very much greater surface area.[1]

Cellular reaction in the host tissues surrounding an autogenous cartilage graft would be completely absent in two months after transfer. Of interest also in the reports is the fact that preserved ox cartilage used for the support of ears did not stand up as well clinically as when similar grafts were used to support saddle nose and facial depressions, where the grafts were better covered by nourishing tissues. This brings up the following question: Do preserved grafts with dead cells require good circulation from tissue fluid to preserve the dead matrix structure of the grafts? One notes that preserved homogenous cartilage grafts are absorbed more rapidly when they are transplanted in avascular burn scar areas. Perhaps the hyaline matrix undergoes chemical changes more rapidly in the absence of adequate tissue fluid even though the chondrocytes have died, and this change may render the matrix substance more vulnerable to absorption by the hostile host tissues.

Mr. John North, from Prof. Pomfret Kilner's Plastic Center in Oxford, England, presented the experiences of the Oxford

[1] I have noted that preserved diced sting-ray cartilage grafts buried in human tissues are absorbed more rapidly than large blocks of sting-ray cartilage transplanted in similar transplantation sites.

CHAPTER 13

Transplantation of Cartilage in Humans (Continued)

HETEROGENOUS CARTILAGE GRAFTS IN HUMANS

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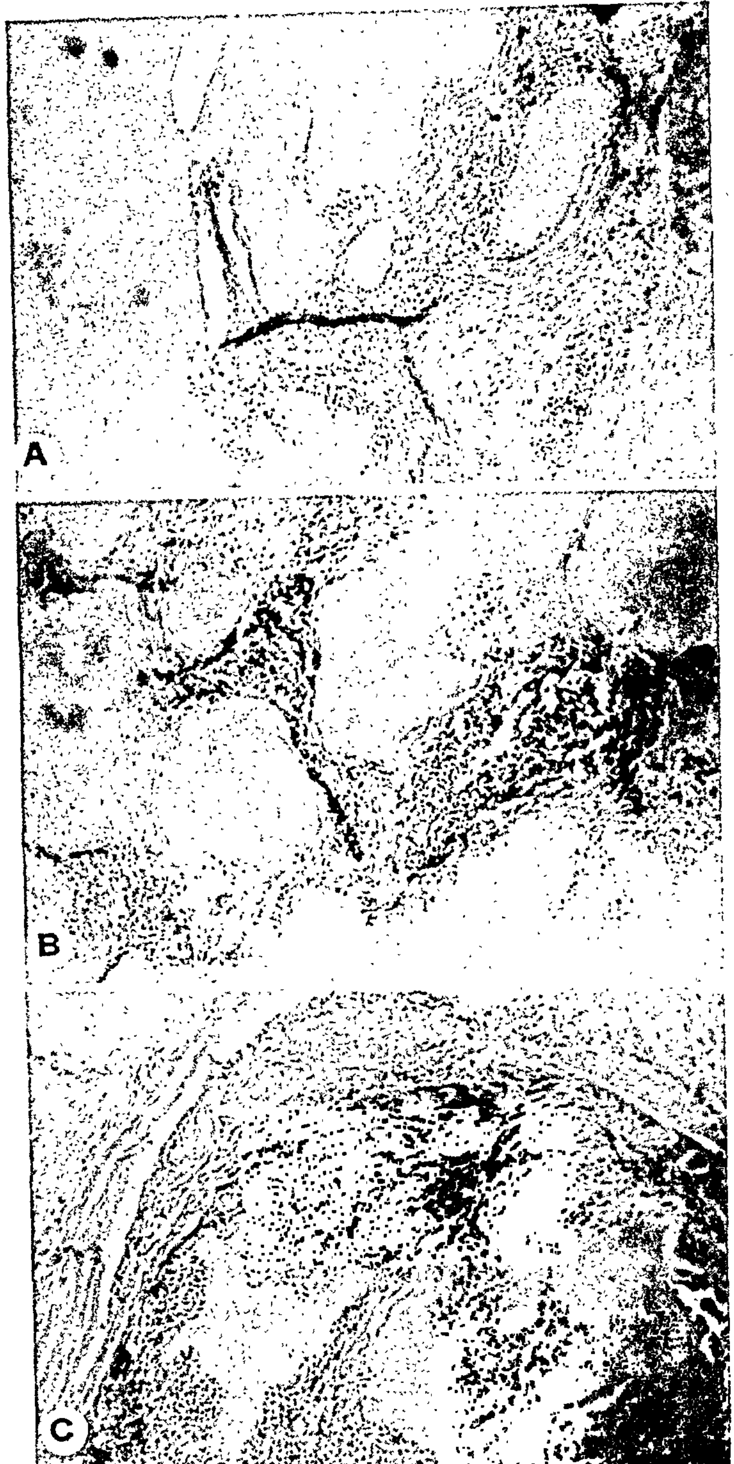


FIG. 35. A. Sting ray cartilage preserved in alcohol and buried in human abdominal fat for 4 months. The host tissues are beginning to invade and absorb the foreign cartilage. In addition to fibroblasts, giant cells, lymphocytes, polymorphonuclear leukocytes, plasma cells and eosinophiles were seen in the area of host tissue invasion.

B. Sting ray cartilage preserved in alcohol and buried for 8 months in human abdominal fat. There is more extensive invasion and absorption of the foreign graft, which is split up into small segments to facilitate absorption.

C. Sting ray cartilage buried for 18 months. Only small remnants of the cartilage remain.

cutting and shaping difficult, but there were occasional vascular channels present in the cartilage.

Measured segments of this ray cartilage were immersed in normal saline for ½ hour and buried in human abdominal fat. The first section, transplanted for 14 months, could not be palpated through the skin and had therefore undergone considerable absorption. Grafts removed in 6 months and 8 months respectively had retained their cartilaginous hyaline structure, with slight reduction in size. Microscopic examination disclosed areas of invasion by host connective tissue and a rather disturbing cellular reaction in the host tissues surrounding the grafts. Both grafts were encased in the usual connective-tissue capsule. The host cells

group with preserved ox cartilage at a recent meeting of the American Society of Plastic and Reconstructive Surgery. Grafts used to repair saddle nose, to reconstruct the ear, and to restore facial contours showed a definite tendency to absorption and replacement by host tissue over a period of about two years, thus indicating that ox cartilage is not entirely suitable as a grafting material in human tissues.

The bold use of preserved ox-cartilage grafts in humans by Sir Harold Gillies is characteristic of this pioneer plastic surgeon, who refuses to grow old, and it presents a challenge to other plastic surgeons and investigators. One ponders the possibility of transplanting fresh heterocartilage grafts with living chondrocytes into human tissue, and what would be the behavior of fresh embryonal heterocartilage grafts in human tissues. As certain bacterial cells such as bovine tubercle bacilli have the ability to survive in both animal and human tissues, a more complete chemical knowledge of this acquirement might be applied to the cartilage cells in zoografts. Thus far there have been no reports on the transplantation of fresh cartilage zoografts in humans.

Gillies, who may aptly be called the father of modern plastic surgery, was the first to report the microscopic findings in human autogenous and fresh homogenous cartilage grafts and also in preserved ox cartilage transplanted in human tissues.

Experiments with Giant Sting-Ray Cartilage

The author decided to transplant heterografts of cartilage from forms genetically very far removed from man, such as the elasmobranch family, which live in the sea. It was conceived that this would be a good test for Borst's (5) and Loeb's (6) theory of genetic relationship and Bacsich and Riddell's theory concerning the protective quality of mucoprotein in the matrix.

Cartilage from the giant sting ray was transplanted experimentally (7) into human abdominal fat and into tubed pedicles of skin. This cartilage was obtained from Mr. F. G. Wood, Jr., the curator at the Marine Gardens in Florida, and shipped to Newark in 70 per cent alcohol at refrigerator temperature. It had been removed from the sternum of a young adult sting ray, weighing 1,000 pounds.

The sting ray belongs to the same elasmobranch family as the shark but, in Mr. Wood's opinion, ray cartilage is preferable because in large sharks the sternum is frequently ossified. The cartilage as received by us from the Marine Gardens in Florida had a beautiful homogeneous texture and was encased in a thin chitin-like armor, which was similar to that found around insect cells. Microscopic examination of control sections revealed an appearance exactly like human hyaline rib cartilage. There were no calcified areas to make

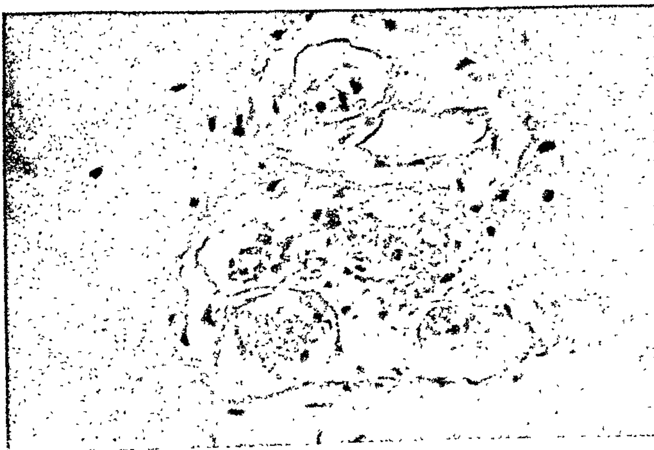


FIG. 34. Control section of sting-ray sternal cartilage showing blood vessels in the cartilage matrix. Ox cartilage also normally contains some blood vessels. The hyaline matrix of sting-ray sternal cartilage in other ways appears exactly like human cartilage excepting that it is covered by a thin hard, chitin-like layer on its external surface ×340.

tissue differentials and Medawar's conception of antigen-antibody reaction fit in this pattern very nicely to explain the behavior of heterogenous, homogenous and autogenous cartilage grafts. Possibly heterocartilage grafts with living cells would persist for about the same time interval as heterocartilage grafts with dead cells.

Heterogenous cartilage grafts are still in the field of experimental surgery, since only early reports on their behavior are available. These early reports, however, indicate a definite and progressive activity on the part of the host tissues to destroy the graft structure.

Drawings Indicating Usual Behavior of Cartilage Grafts in Man

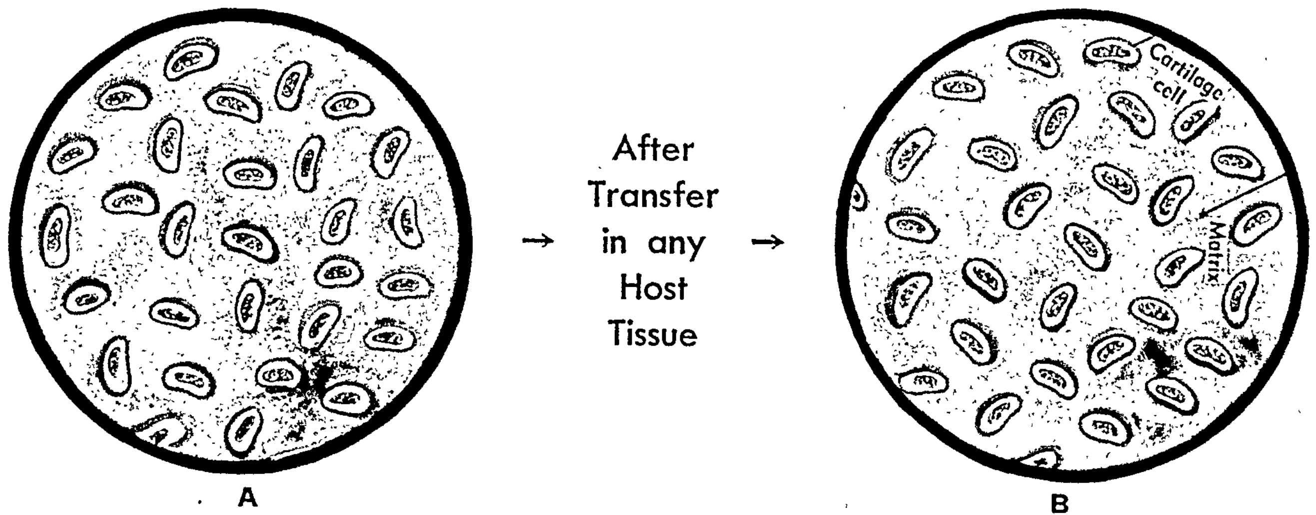


Fig. 37. A. Fresh autogenous human cartilage grafts. B. The grafts remain as cartilage. The cartilage cells remain viable and the matrix tends to retain its specific structure (hyaline and elastic).

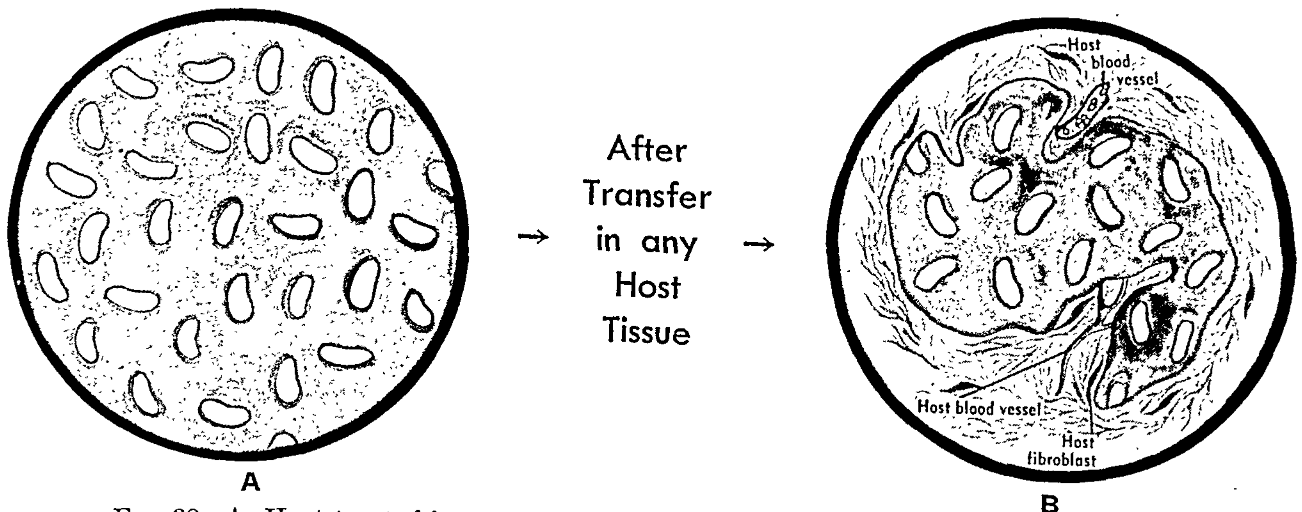


Fig. 38. A. Heat-treated human autogenous cartilage grafts. B. The graft structure is slowly invaded and replaced by fibrous tissue.

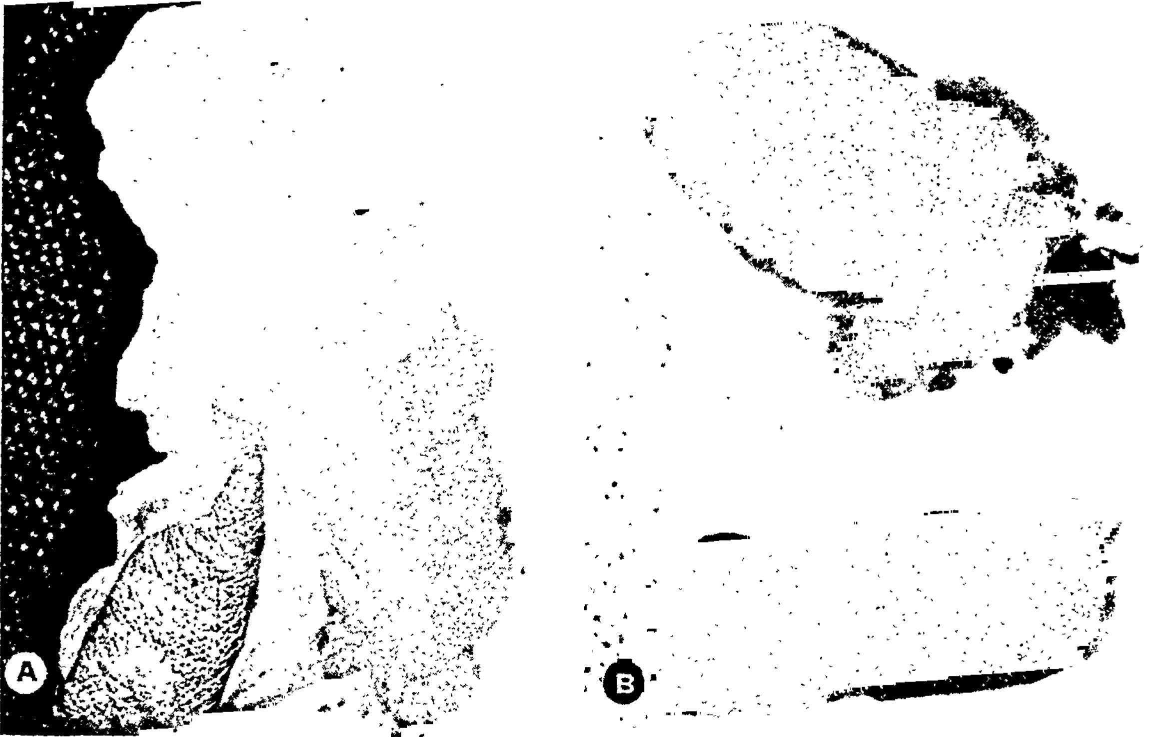


FIG. 36. A. Sting-ray cartilage graft preserved in alcohol and buried for 1 year in human abdominal fat. Note thick fibrous capsule adherent to the partially removed foreign graft. B. Fibrous sac and sting-ray cartilage graft after complete removal. The graft was reduced by about ⅓ of its original bulk by actual measurement and showed numerous areas of invasion by host tissues.

surrounding the graft were about 50 per cent lymphocytes and 50 per cent assorted cells. The latter were identified as eosinophiles, blue-staining plasma cells with characteristic cart-wheel nuclei, polymorphonuclear leukocytes, histocytes and occasional foreign-body giant cells. In a section buried for 14 months, narrow strands of host fibroblasts accompanied by blood vessels had penetrated the graft structure and separated it into numerous islands resembling diced cartilage grafts. Apparently the plan was to expose a large surface area of cartilage before the infiltration of host cellular elements which will be associated with large-scale absorption of the foreign cartilage. After the cartilage had been separated into numerous isolated cartilage islands, plasma cells, eosinophiles, polymorphonuclears, lymphocytes, histocytes and giant cells migrated to the area and large scale absorption took place as shown in later sections.

Summary Comment

The available evidence regarding heterogenous cartilage transplantation confirms Borst's and Loeb's theory of tissue specificity as affected by genetic relationship. The autograft is well adapted to altered location, the homograft to a less extent; the heterograft, with its more remote donor-recipient relationship, excites a still greater reaction in the host tissues, which will eventually invade and destroy the graft.

The long survival time of the matrix of heterocartilage grafts contrasted to that of many other heterogenous tissues may be owing to the mucoprotein interstitial substance between the dead chondrocytes, which serves as a sort of buffer between the graft cells and the host (8). Loeb's theory of

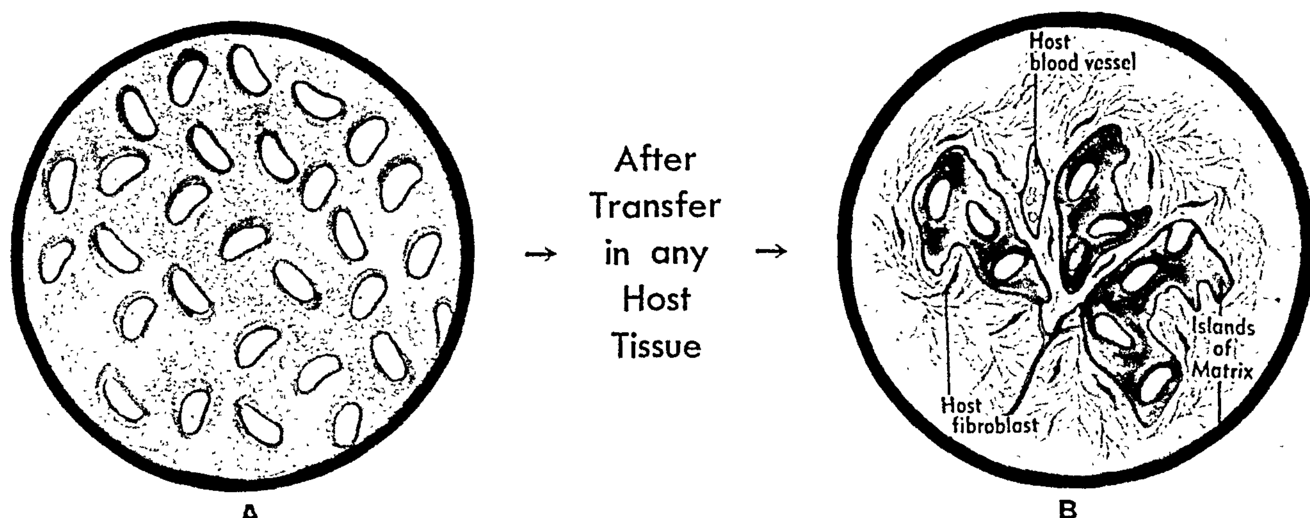


Fig. 41. A. Preserved heterogenous cartilage grafts. B. The graft is invaded and replaced by fibrous tissue.

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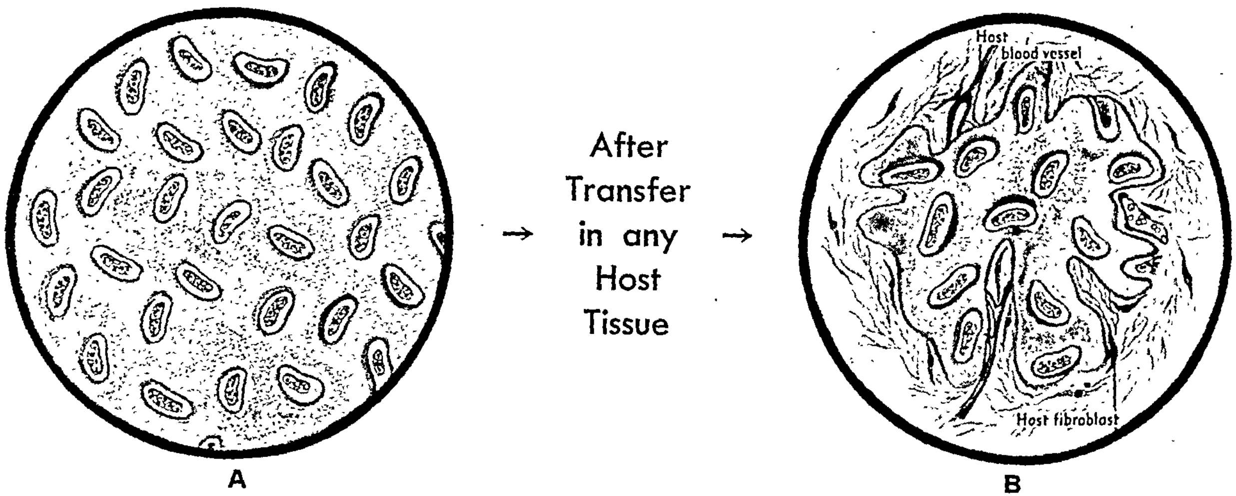


Fig. 39. A. Fresh homogenous human cartilage grafts. B. The matrix is gradually invaded and absorbed by the host tissues. The cartilage cells in the graft remain viable until their matrix has been removed. They then disappear among the large numbers of host fibroblasts.

After Transfer in any Host Tissue

New bone formation

New bone

Host blood vessel

A

C

Host Fibroblast

Host blood vessel

Host blood vessel

B

Fig. 40. A. Homogenous human preserved Cartilage grafts. B. Graft is gradually invaded and replaced by fibrous tissue. Occasionally new bone formation is seen within disintegrating graft or near its outer surface as in C.

if any place in clinical surgery at this time. Ox cartilage preserved in merthiolate solution has been used as grafting material with varying degrees of success (2–5). The careful experimental studies regarding the fate of ox cartilage in human tissues by Gibson and Davis indicate that the cartilage acts as an antigen, and that the intensity of the antigen-antibody reaction increases in direct proportion to the duration of the host's exposure to this foreign protein. Preserved cartilage from the giant sting ray (6) transplanted in human tissues seems to stimulate a local cellular reaction, and a possible antibody reaction in the host tissues which results in destruction of the foreign cartilage graft.

In general, all foreign grafts are believed to stimulate an immune response in the host tissues, and this immune response is detrimental to the survival of various homogenous tissue grafts over different periods of time, as demonstrated by Medawar (7). Fresh homogenous grafts of cornea, lens, and cartilage resist absorption, and the cells remain viable,[2] because these tissues are avascular and because their gel-like matrix is rich in mucoprotein, which serves to protect the cells from hostile host antibodies (8).

Alternately, in tissues such as homogenous skin grafts, homogenous fat grafts and homogenous kidney transplants, which are vascular and not sufficiently protected by gel-like mucoprotein, the cells are destroyed in short periods of time presumably by the hostile host antibodies, and the graft matrix disintegrates. The heterograft, being foreign, fares even worse than the homograft (9).

Growth of Cartilage Grafts

Normal growth of all cartilage structures during childhood takes place from the deep layer of connective-tissue cells of the perichondrium. These cells presumably form a matrix substance about themselves, separate from the perichondrium, and become cartilage cells (appositional growth). Growth also occurs by division of cartilage cells, followed by the deposition of matrix about each cell separating one from another. After adult life, cartilage ceases to grow, and there is considerable doubt concerning its powers of regeneration following injury; the cartilage wound being filled in by connective tissue associated with little, if any, new cartilage formation.

Adult autogenous cartilage grafts retain their cartilaginous structure following transplantation, but usually neither increase nor decrease in size. Dupertuis (10) in 1941 demonstrated actual growth of young autogenous and homogenous cartilage grafts in rabbits, and I reported evidence of growth in some young autogenous human cartilage grafts (11). Later, removal of young autogenous rib-cartilage grafts buried up to seven and a half years failed to show any increase in size (12). One is inclined to believe that the growth factor is absent in young human cartilage grafts. At any rate it does not occur consistently and cannot be depended upon from a clinical standpoint.

NASAL DEPRESSIONS

Rib cartilage grafts were used by von Mangoldt (13) in 1899 to support saddle nose and this method is still employed today, although some surgeons prefer bone grafts.

For the repair of nasal depressions it is

[2] A fresh homogenous human cartilage graft which had been transplanted from an infant to a young child was sectioned in the fresh state, and the cells were found to be viable as evidenced by supravital dye stain. In areas where the matrix had been absorbed the cartilage cells were absent, but cells protected by only a thin layer of matrix were living cells. Pre- and postoperative measurements demonstrated that the graft was reduced in bulk. The graft was removed four years after transplantation.

CHAPTER 14

Clinical Use of Cartilage Grafts

Of all buried grafts cartilage is probably the most widely used. It is unique in that it adjusts itself to almost any tissue environment, provided it is completely surrounded by nourishing host tissue and not exposed. Unlike iliac and rib-bone grafts, it survives equally well whether in contact with like tissue (cartilage) or some other tissue such as fat, muscle, or fascia.

The available clinical and experimental evidence regarding the fate of the various cartilage grafts should enable a surgeon to choose rather definitely between autogenous and homogenous cartilage according to the requirements of any given case.

Choice of Cartilage Type

Fresh autogenous cartilage is always the material of choice for transplantation. The cells in autogenous cartilage grafts survive transplantation as living cells[1] which continue to maintain and service their intercellular matrix. Occasional grafts may be partly absorbed, especially in children or in avascular transplantation sites, but the amount of absorption is less than that which occurs in homogenous grafts for a particular individual. Curiously, one notes that preserved homogenous grafts may be completely absorbed in avascular scarred sites, whereas autogenous grafts tend to remain when transplanted in the same locality at a second operation.

Homogenous cartilage, either fresh or preserved, is a valuable second choice when it is not expedient to remove the patient's own cartilage (1).

Autogenous cartilage is especially indicated in children and young adults, who have a long life expectancy and therefore require longer-lasting grafts than do elderly patients. When large amounts of cartilage are required, as in the reconstruction of both ears in young children, cadaver cartilage may be used together with as much autogenous cartilage as can be obtained.

The cells in fresh homogenous cartilage grafts survive transplantation as living cells but the matrix is slowly absorbed by host fibroblasts and the exposed chondrocytes disappear. Clinically, preserved homogenous grafts retain their structure about as well as fresh homogenous cartilage grafts but bank cartilage is preferred because it is more readily available. Absorption and replacement by connective tissue or bone may occur in both autogenous and homogenous cartilage grafts, but the tendency is greater in the foreign graft.

Heterogenous cartilage grafts have little

[1] Autogenous rib, septal and ear cartilage grafts buried up to 27 years have been sectioned in the fresh unfixed state and stained with supravital dyes. The chondrocytes in all these grafts were viable cells.

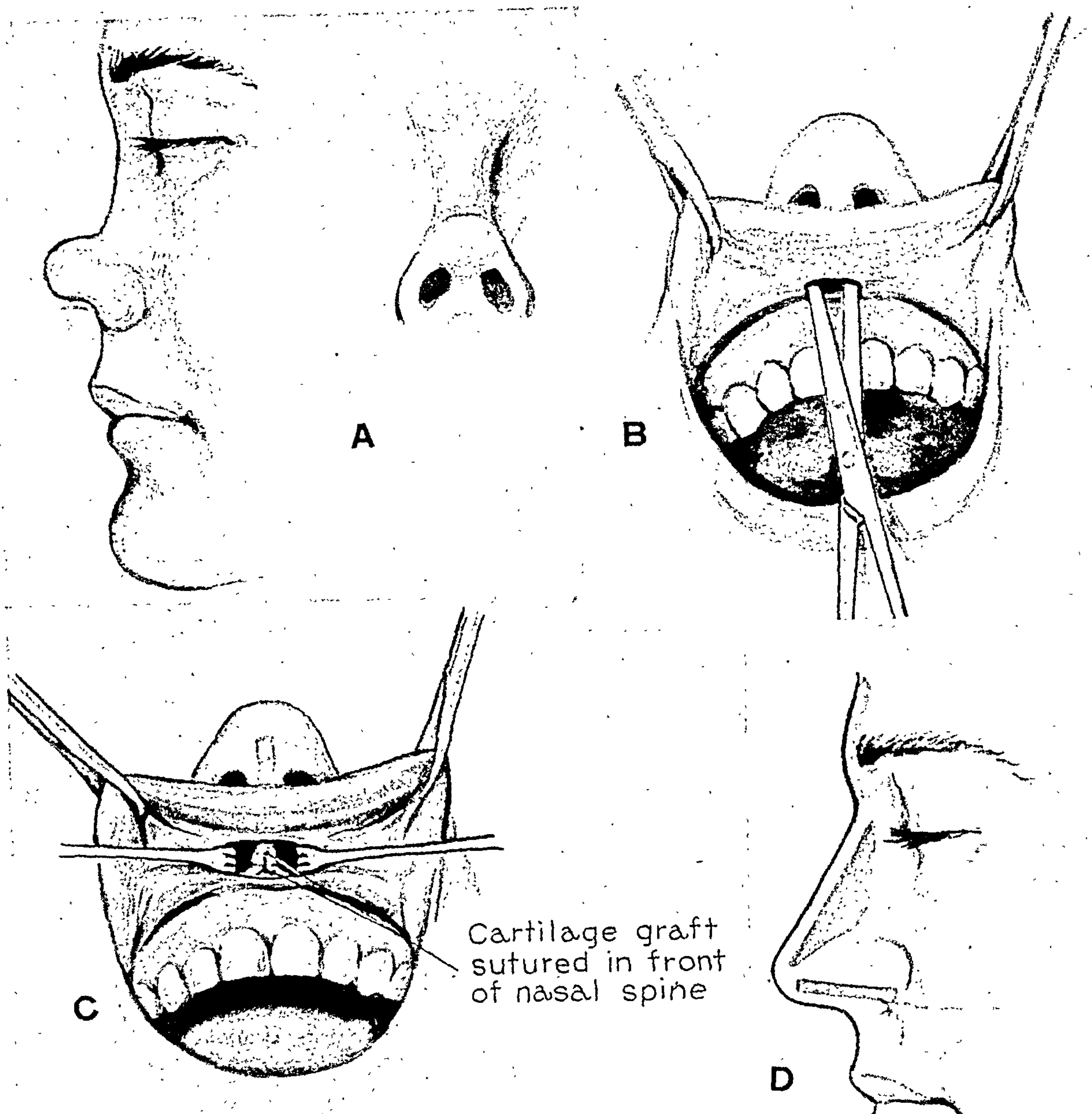


FIG. 43. A. Saddle nose deformity with retracted columella.

B. A subcutaneous tunnel made in the columella through an incision in the mucous membrane.

C. Cartilage graft inserted in columella to support tip of nose. Wide undermining of mucous membrane covering septal cartilage permits the insertion of a thick cartilage graft to bring the columella downward, so that it is lower than the rim of the nostril margins. Base of cartilage sutured to nasal spine.

D. Lateral view after a second cartilage graft has been inserted through an intranasal incision to correct the saddle deformity. Each graft is separate.

always seems to adjust itself to the tension of the nasal skin and to provide an even soft contour.

Occasionally one sees nasal fractures with depressions on one or both sides of the nose just below the bony arch. This is due to separation of the lateral cartilage from its normal attachment to the nasal bone. Such depressions can be nicely filled with an alar cartilage graft.

Fortunately it is nearly always possible to remove the posterior three-fourths of an

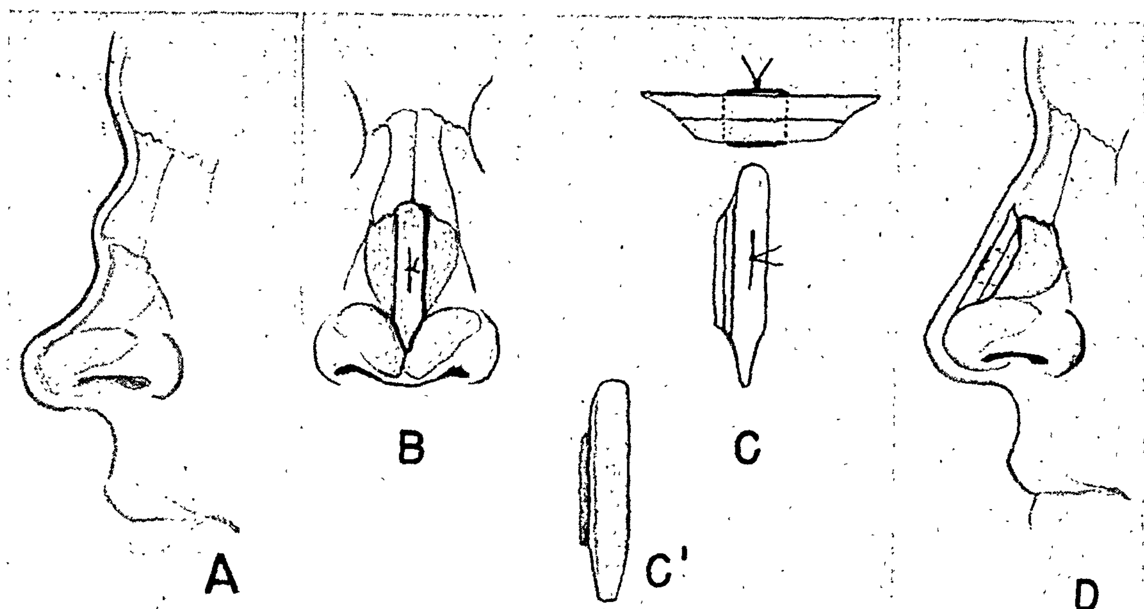


Fig. 42. A. Saddle deformity of the lower nose such as is often seen after an injury, septal abscess, or too radical submucous resection operation.

B. Two segments of the patient's septal cartilage sutured together have been inserted through an intranasal incision to correct the saddle deformity.

C. Shape and relationship of composite graft which must accurately fit the contours of the base on which it rests.

C'. Composite septal cartilage graft capped with an overlapping alar cartilage graft to provide a smooth contour.

D. Septal cartilage capped with alar cartilage is inserted through intranasal incision to fill saddle deformity.

wise to introduce the cartilage through a concealed incision made within the nasal cavity, thus avoiding a visible scar. If autogenous cartilage, which is the best grafting material, is to be used, the surgeon should bear in mind that any type of autogenous cartilages may be utilized.

When the defect is small, septal cartilage, ear cartilage, and alar cartilage are readily available. Rather deep depressions in the nose can be adequately filled with a composite graft consisting of several segments of septal cartilage capped with a rather broad alar cartilage graft. The alar cartilage bends convexly in conforming to the tension of the nasal skin, and thus serves adequately as a smooth covering for the top and sides of the composite graft.

Septal bone (14) may be used to supplement the septal cartilage when additional bulk is required, or septal bone capped with alar cartilage can be employed when septal cartilage is absent.

Alar cartilage in single or multiple layers may be used to restore the dorsal line of the nose when too much nasal bone or septal cartilage has been removed during a rhinoplasty. Because of its thinness and flexibility, alar cartilage is an excellent grafting material for restoring the contour of the nostril in secondary rhinoplasties to correct the pinched-in depression at the side due to excessive removal of alar cartilage at the time of the original operation.[3] Flattened areas at or above the nasal tip and clefts in the columella may also be repaired with this thin malleable type of cartilage, which

[3] Preserved alar homografts may be used when sufficient autogenous alar cartilage is not available in such patients.

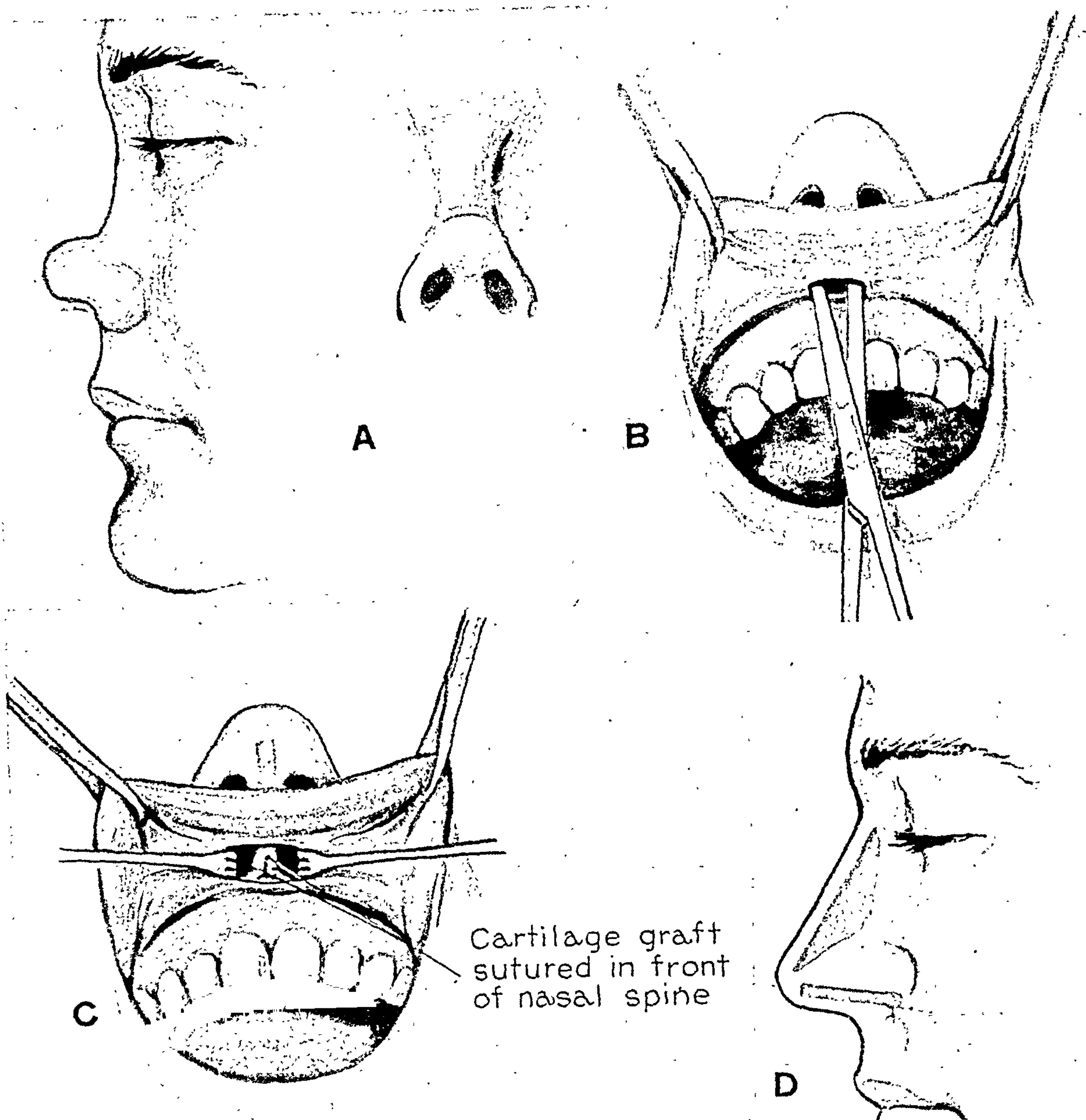


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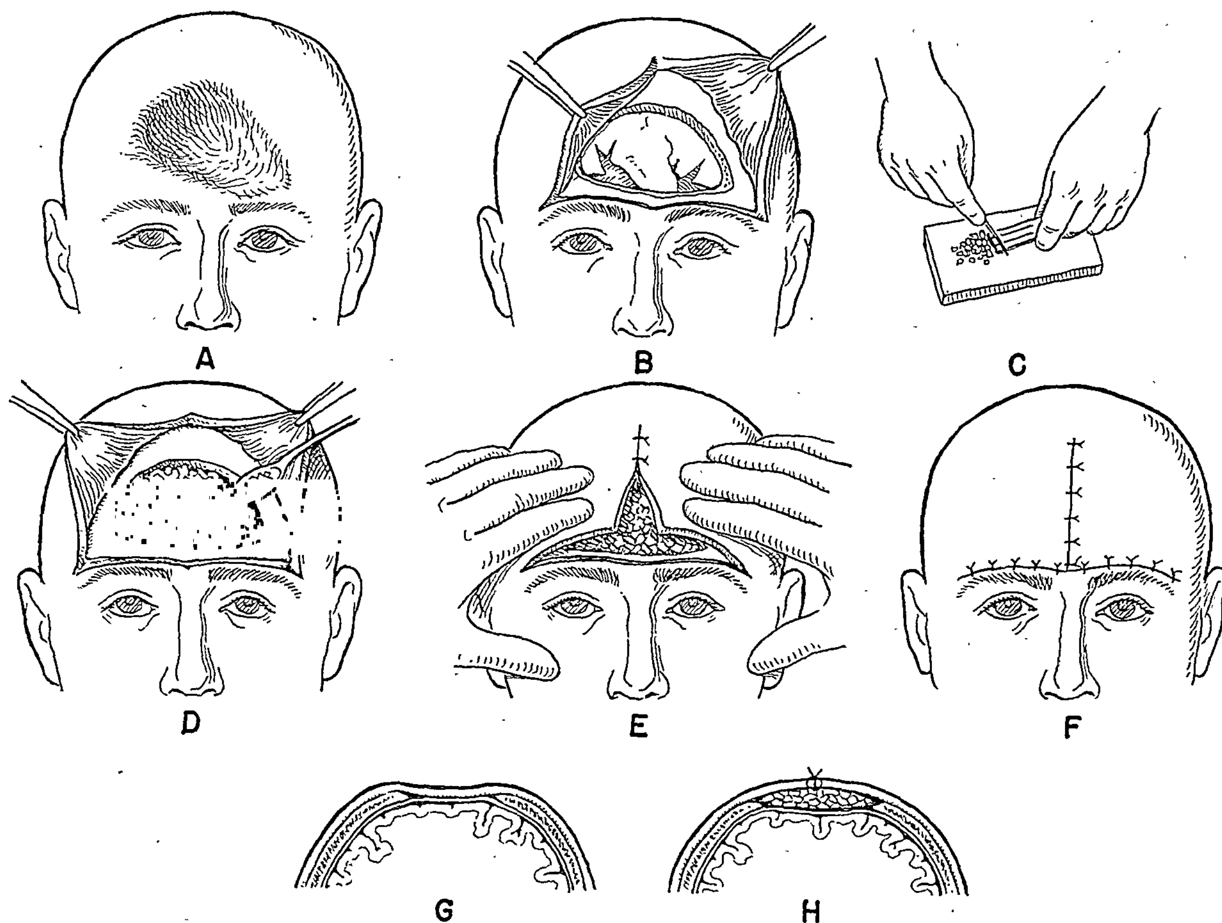


FIG. 44. A. Drawing represents patient with deep depression in frontal area, the skin and scar lying directly over the dura.

B. Scalp skin elevated, exposing defect in bone. The brain pulsates beneath the covering of dura and scar tissue.

C. Pieces of rib cartilage removed from right side of patient's chest are diced into many fine segments.

D. The diced cartilage grafts are introduced over the exposed dura with a spoon.

E. The mass of diced-cartilage grafts is patted into a smooth, even contour like wet grains of sand.

F. The inverted T-incision is sutured over the rounded mass of diced-cartilage grafts.

G. Cross-sectional view of defect.

H. Cross-sectional view of defect, with diced-cartilage grafts in place.

From Peer, L. A.: Diced cartilage grafts. Arch. Otolaryngol., 38: 156 (Aug.) 1943.

alar cartilage without producing any deformity at the nasal tip. Consequently this area may be regarded as a donor site for alar cartilage, as the ribs provide rib cartilage and the nasal septum, septal cartilage.

Use of Rib Cartilage Graft

Deep saddle depressions in the nose can be repaired by rib cartilage, which is available in sufficient bulk to fill the cavity adequately. There are several points in the technique of employing rib cartilage grafts for nasal depressions that are sufficiently important to mention.

In the first place it is essential to shape the graft so that it conforms evenly with the contour of the tissues on which it rests. A straight piece of rib inserted like a beam of wood is bound to become distorted due to numerous dead spaces between the

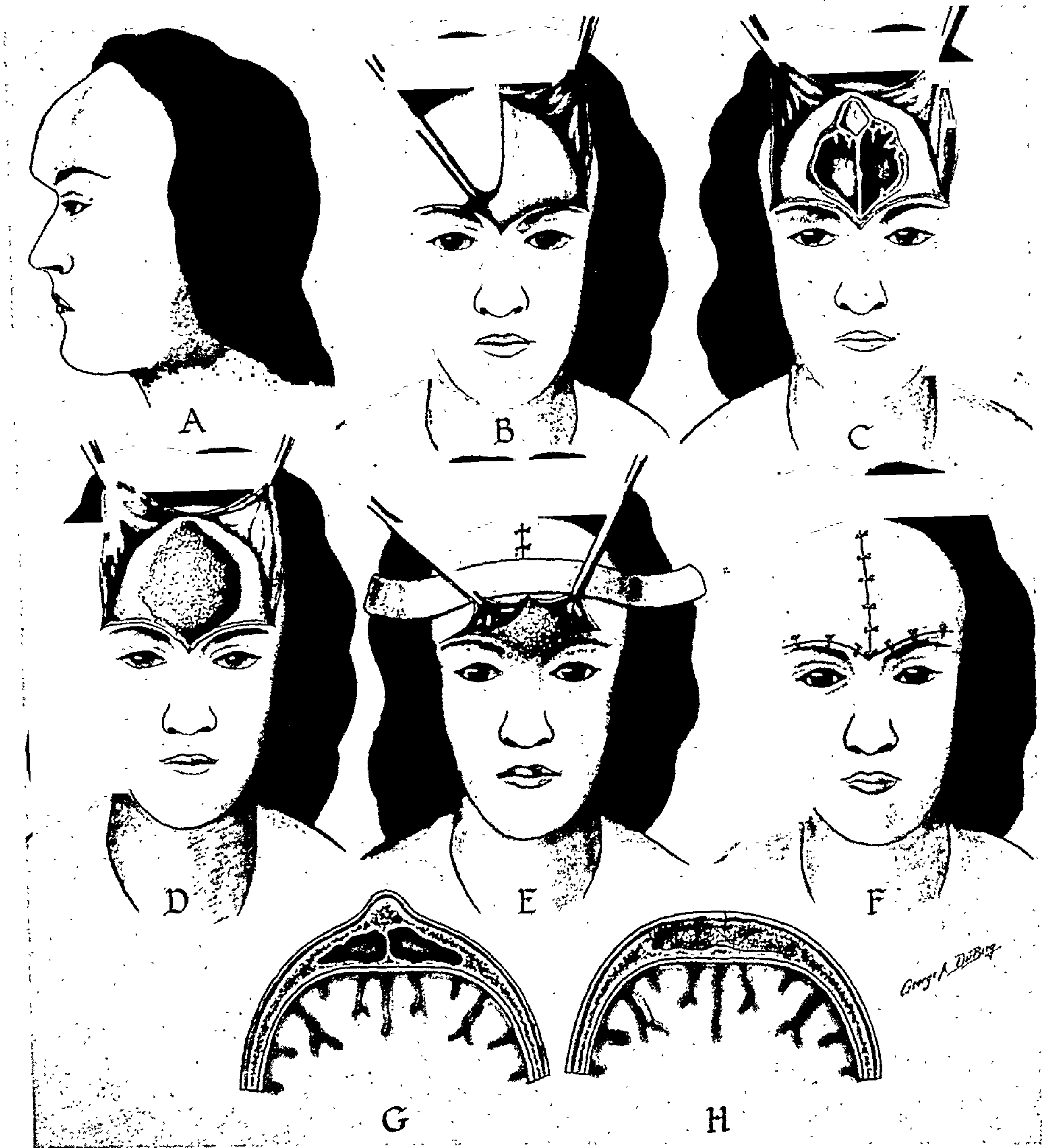


FIG. 45. Repair of defect following removal of osteoma in frontal region of skull with diced-cartilage grafts.

The entire outer table of the skull should be removed as well as the mucous membrane lining the frontal sinus. The frontal-nasal duct is curetted and diced cartilage introduced into the cavity.

surface of the graft and the tissues beneath. Furthermore, this straight beam of cartilage will give a stiff unnatural appearance to the nose and elevate the alar cartilages so that a beak effect is produced at the nasal tip.

After the cartilage has been shaped so that it fits snugly, conforming to basal contour lines, the upper portions of the graft resting on the bony bridge should be cross-cut in numerous places to prevent upward or lateral curling of the cartilage postoperatively. If there is a tendency to prominence about the nasal tip, the graft should be shortened or the posterior por-

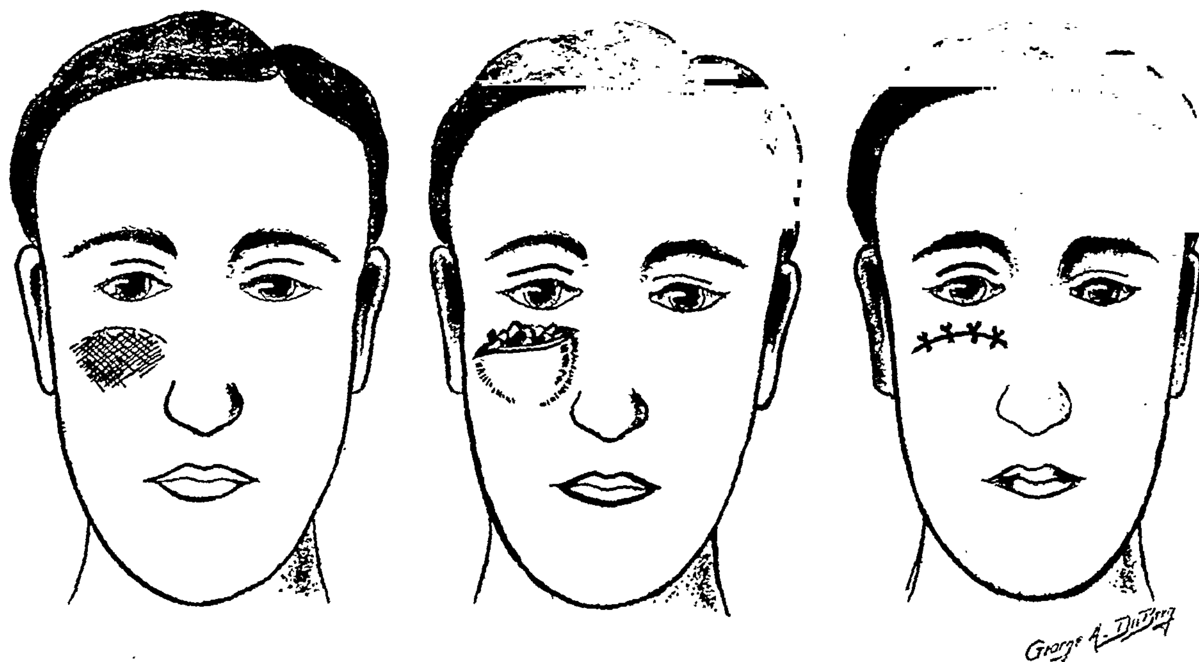


FIG. 46. Drawings illustrating how a depression over the malar bone may be repaired with diced-cartilage grafts. Depressions of the bony floor of the orbit and depressions in the mastoid may also be filled with diced-cartilage grafts. From Peer, L. A.: Arch. Otolaryngol., 38: 156, 1943.

tions of the alar cartilages be removed, as in rhinoplasty.

When a cartilage strut is required in the columella to support the nasal tip, *it should be inserted through a separate incision in the buccal mucous membrane in such a manner that this columellar graft lies in front of the dorsal beam of rib cartilage.* This positioning will nearly always provide a more normal-appearing nasal tip than the procedure of inserting the columellar strut under the dorsal beam of the cartilage.

If the columella is retracted, the base of the columellar graft can be brought downward and held in place by a catgut suture fixing it to the maxillary bone in front of the nasal spine. If the columella is excessively retracted and bound down by scar tissue, the surgeon can obtain relaxation by widely elevating the mucous membrane flaps on each side of the nasal septum so that the graft in the columella can be brought downward. In severe cases of retracted columella it may be necessary to make relaxing incisions through each elevated mucous membrane flap. The columella must be at a lower level than the free border of each nostril to avoid the appearance of a snubbed nose, which often occurs after excessive removal of the septal cartilage in rhinoplasty. Incisions in the skin surface of the columella through which a rib graft may be inserted are not advisable because of external scarring.

CARTILAGE STRUT IN BILATERAL CLEFT LIP

The late postoperative results following the repair of severe bilateral harelip are poor throughout the world. In most of the patients the critical surgeon will be distressed by the position and relationship of the wabbly premaxilla, and the short columella, which gives a bird-beak appearance to the tip of the nose. If the surgeon has performed the columellar lift procedure, the lip is often tight and scarred and the columella is wide, flabby and unnatural in appearance.

The author was so disappointed after

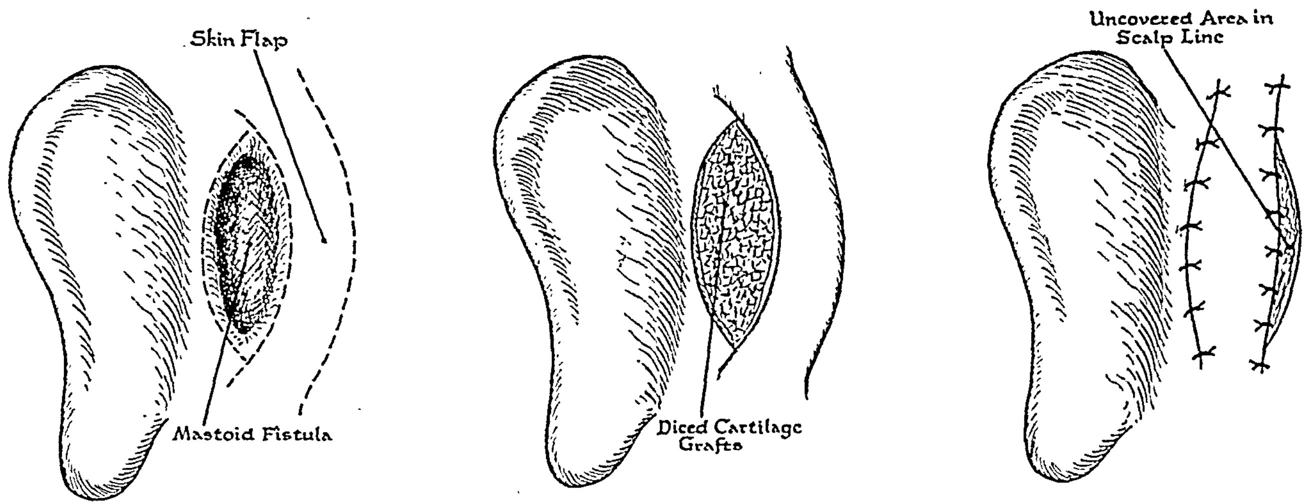


FIG. 47. Drawings illustrating repair of mastoid fistula with diced-cartilage grafts.

examining 100 of his own late double-harelip patients postoperatively that he decided no more extensive columellar lift procedures should be performed by members of the Plastic Department at St. Barnabas Hospital. Instead, we now routinely insert a strut of the child's own rib cartilage, through a buccal mucous membrane incision, to support the nasal tip. The base of this cartilage strut rests on, or lies in front of, the nasal spine, and the other end of the strut supports the nasal tip. At yearly intervals the base of the strut is exposed, the cartilage freed and elevated from the nasal spine and a second smaller segment of cartilage inserted between the spine and the base of the first cartilage graft. This procedure serves to further elevate the nasal tip and lengthen the columella.

It would be very nice if this human cartilage strut grew like autogenous cartilage grafts in rabbits; but in the author's experience one cannot depend on the growth principle of cartilage in humans, and for this reason additional cartilage is inserted at intervals to increase the height of the strut. It is hoped that the continuous tension exerted by the cartilage strut will stimulate growth of the alar cartilages and of the soft tissues. The early results have been very satisfactory, and no infections have developed.

OTHER FACIAL CORRECTIONS

Rib cartilage grafts are used also to elevate the depressed nostril floor often seen in wide unilateral harelip, to build out the contour of the jaw bone in atrophy of the face, and to restore the contour of the malar bone, zygoma, and bony brow.

One or several segments of rib cartilage can be employed to elevate the eye when it has prolapsed downward due to a depressed fracture of the bony orbital floor. This elevation of the eye will often lessen the distressing double vision with which such patients are afflicted. Further improvement may be obtained by the use of prism lenses. Retruding chin can usually be corrected by inserting cartilage grafts over the bone to increase the prominence of the chin. Small degrees of recession can be improved by the insertion of septal cartilage supplemented by septal bone. One should carefully evaluate the occlusion in severe cases of receding chin and have a consultation with an orthodontist to determine the advisability of setting the jaw forward rather than merely adding to the prominence of the chin. After the lower jaw has been set forward as far as possible to obtain better occlusion, the remaining degree of receding chin can be corrected by inserting cartilage to give additional prominence.

In ankylosis of the jaw the ascending

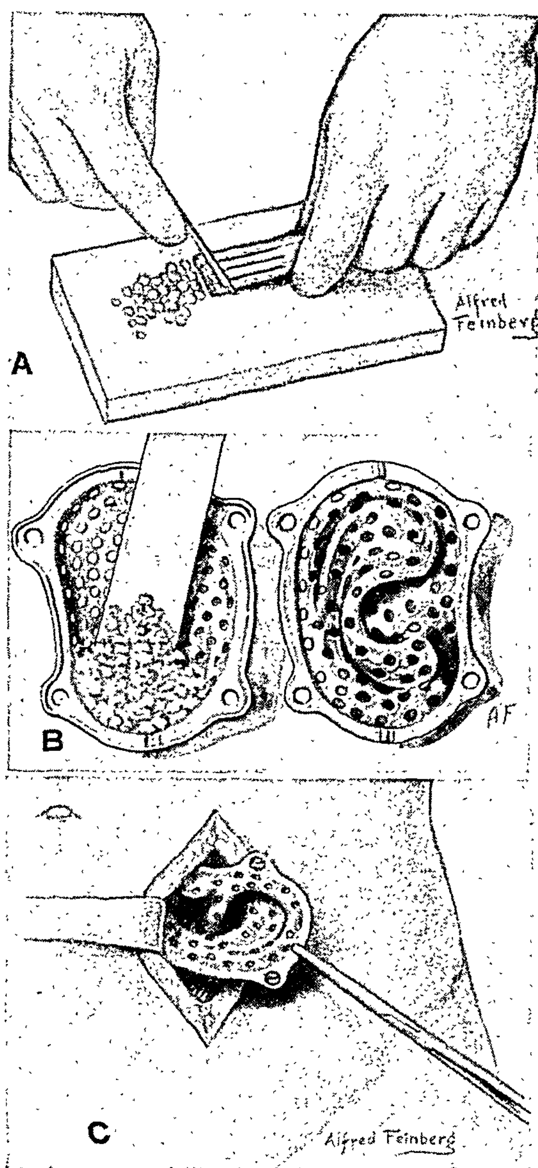


FIG. 48. Formation of ear framework from diced-cartilage grafts inserted in vitallium ear mold.

A. Rib cartilage removed from the right side of the patient's chest is diced into many small cartilage segments.

B. The "diced-cartilage grafts" are introduced into each half of a perforated vitallium ear mold.

C. The two halves of the ear mold have been fastened together with vitallium screws, pressing the diced-cartilage grafts into the shape of an ear.

The mold containing the cartilage segments is inserted in a pocket beneath the patient's abdominal skin. During a period of months, blood vessels and connective tissue grow through the openings in the mold and fasten the separate cartilage segments firmly together in the form of an ear.

When both auricles are being reconstructed in a young child, diced cadaver cartilage is used to supplement the child's own cartilage so that there will be sufficient cartilage to fill both the right and left ear molds. The operative procedures for the right and left ear are carried out simultaneously. Thus, the total number of operations for reconstructing two auricles are the same as for reconstructing a single auricle.

The vitallium ear molds are made by the Austenal Company of New York City. Medium and large sized models are available to meet the general requirements of individual cases. (21.)

ramus is often rather short, and removal of a segment of bone to provide a false joint produces further shortening. A segment of cartilage about the size of the removed bone may be inserted between the detached upper and lower bony segments to prevent shortening of the ramus. If the cartilage graft is sufficiently large, the length of the ramus may be actually increased, as suggested by Dufourmentel and Darcissac (15). Braithwaite and Hopper (16) have used preserved ox cartilage for this purpose.

In general, one feels that cartilage as an onlay graft tends to retain its bulk and contour better than rib and iliac bone. Bone grafts inserted between severed bony segments to restore continuity and function, especially where there is movement as in the jaw, or movement and weight-bearing as in the tibia, appear to retain their structure quite well. When rib and iliac bone grafts are applied as simple onlay grafts in contact with the jaw to restore contour, and in the brow or skull to build out depressions, the grafts often are gradually reduced in size. Contrariwise, for saddle depressions in the nose, bone grafts from the rib and ilium appear to retain their general bulk effectively, according to the surgeons who use them for this purpose (17, 18) even though the grafts are not in actual contact with the nasal bones. Rib and iliac bone grafts not in contact with bone elsewhere in the body are absorbed and replaced by fibrous tissue. Thus it appears that the nasal tissues constitute a specially favorable transplantation site.

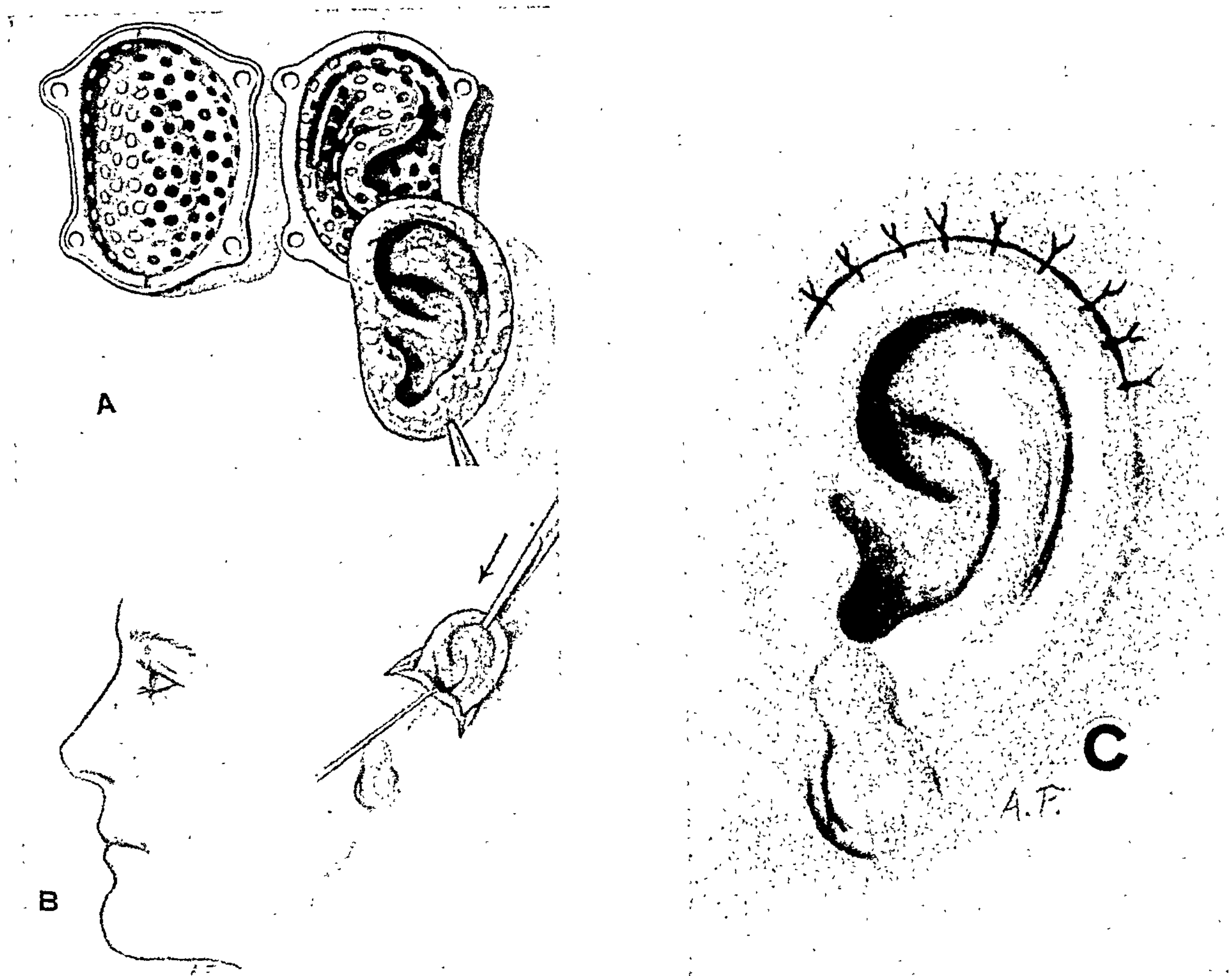


FIG. 49. Removal of diced cartilage ear from mold and transplantation in ear region.

A. The vitallium ear mold has been removed from the abdominal pocket 5 months following burial. The two halves of the mold have been separated and the diced-cartilage ear framework removed from the mold.

B. The diced-cartilage ear framework is inserted in a previously delayed pocket beneath the skin in the ear region. The framework is about 1 inch higher than the normal ear to allow for subsequent sagging.

C. The wound edges are sutured and a firm dressing applied to press the skin against the cartilage framework. A drain may be inserted in the lower angle of the wound. The drain is removed after 48 hours without disturbing the firm dressing holding the skin against the cartilage. (21.)

DICED-CARTILAGE GRAFTS

Diced-cartilage grafts (19), as the name suggests, consist of numerous small segments of cartilage which can be packed or molded into any desired contour like wet grains of sand. The term "diced" is descriptive because in early cases the grafts were obtained by pinning a rib cartilage segment to a wooden block, making linear cuts in the cartilage, and then cross-cutting the rib, much as a cook dices carrots in preparing a salad. In practice, however, the small segments were used in the form of flat shavings rather than cubes, because the thin shavings pack together without presenting sharp corners, which may produce a prominence beneath the overlying skin.

Use in Skull

Diced-cartilage grafts were first used by the author in skull depressions, the multiple segments being introduced in the cavity, and gently patted in a rounded contour. The scalp skin was then sutured over the

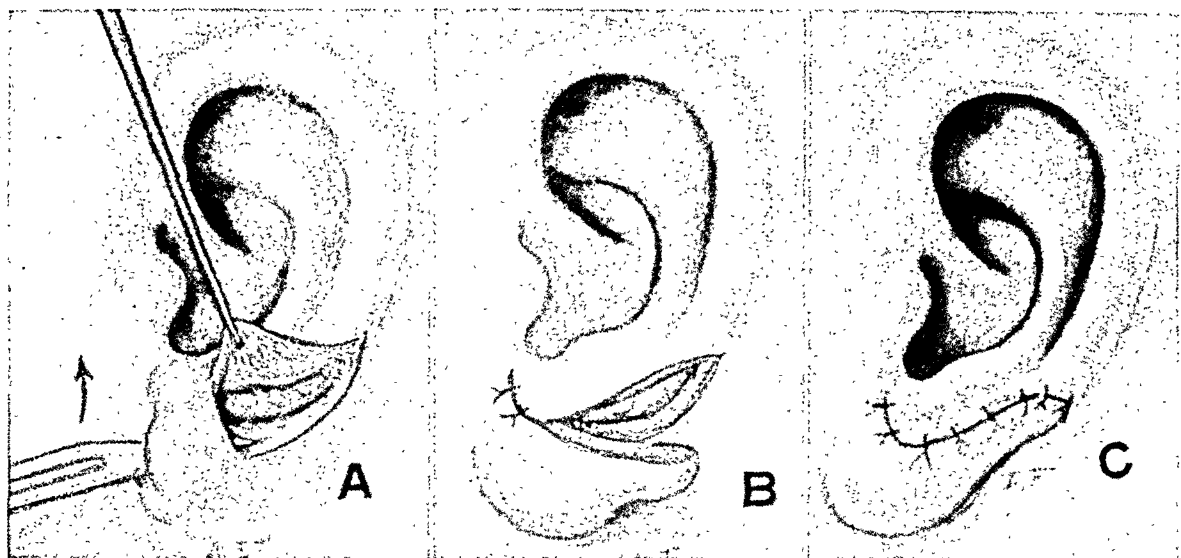


FIG. 50. Adjustment of ear lobe into normal relationship with reconstructed auricle.

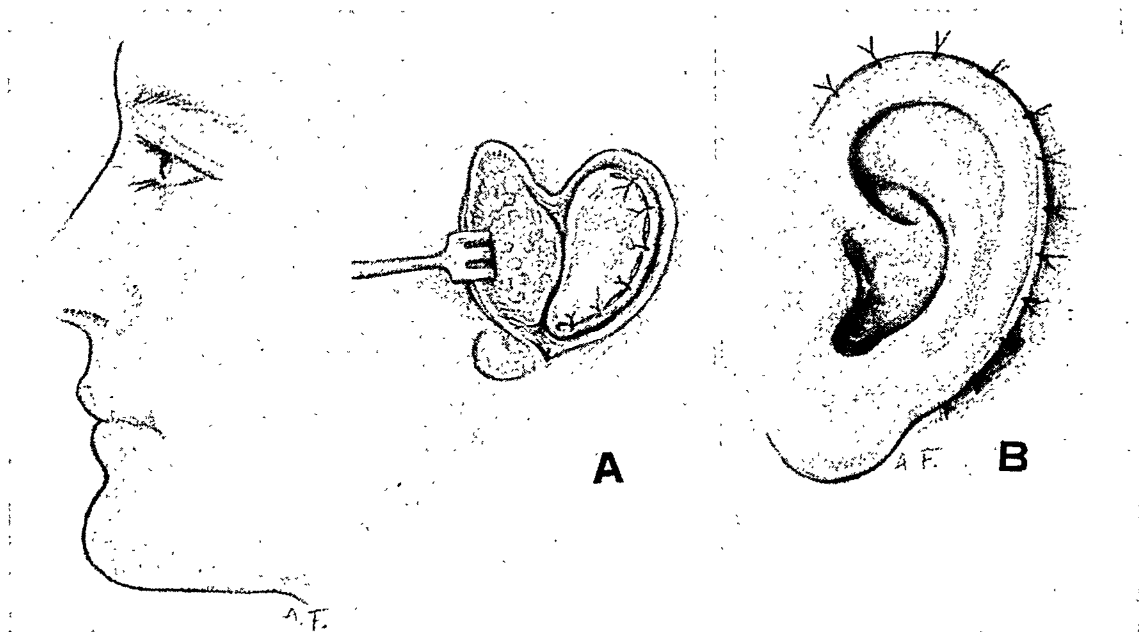


FIG. 51. Grafting posterior surface of auricle and raw scalp surface with split skin graft.
A. The diced-cartilage framework is dissected free from the temporal fascia and periosteum of the temporal bone. A split skin graft sutured over a dental stent mold is inserted behind the new auricle to cover the back of the diced-cartilage framework and the raw scalp surface.

B. The auricle is sutured over the dental stent mold, which is covered with split skin graft. After about 7 days, the dental stent is removed. The split skin graft covers the raw scalp area and the posterior surface of the new auricle, permitting the latter to stand out at an angle from the side of the head. The degree of protrusion and height of the reconstructed auricle must be adjusted to conform with the normal ear on the opposite side of the head.

rounded surface of the cartilage mass. This method provides a smooth, even contour and is so simple in execution that, in the hands of a good operating team with good operative assistance, a large depression in the frontal region can be repaired in an hour.

Microscopic examination of the diced-cartilage grafts following transplantation at various intervals up to four years has been made. The sections demonstrated that the spaces between the small cartilage segments were occupied first by blood and later by ingrowing connective tissue accompanied by numerous small blood vessels. Each small cartilage graft rested against adjoining cartilage at some points (with a thin layer of connective tissue between), thus preventing contracture of the cartilage mass. The bulk of the diced-cartilage grafts collectively was increased by the addition of the numerous small spaces between the cartilage, and for this reason the diced-cartilage grafts actually filled a larger space than the solid rib cartilage from which they were cut.

Autogenous diced-cartilage grafts had living chondrocytes, normal-appearing matrix with occasional bone formation in or outside the grafts, and a general absence of invasion and absorption. Preserved diced-cartilage grafts showed definite invasion and absorption in some grafts, whereas in others the matrix, with dead cells, appeared to resist invasion quite well. The tendency to bone formation was more pronounced in the preserved cartilage than in the autogenous cartilage.

Diced-cartilage grafts, in the author's opinion, have a field of usefulness in repairing depressions in the frontal region of the skull which is equal to any other method in use at the present time. From a theoretical standpoint the effectiveness of bone grafts should be greater than that of cartilage, because in using bone the surgeon is replacing an absent tissue with a similar one. Late examination of skull depressions repaired with iliac bone demonstrates considerable absorption in some cases, but the reduced bone graft appears to have bony contact with margins of the skull bone and thus provides protection for the brain. Some neurosurgeons hold that autogenous grafts of skull bone do not make bony contact with the adjacent skull bone of the host when used as free grafts. Gilbert Horrax of the Lahey Clinic believes that only a fibrous union occurs in such patients, but Francis Grant holds that an osseous union is formed (20).

Tantalum plates and other foreign bodies have a field of usefulness in elderly patients with skull defects and in cancerous patients with doubtful prognosis. Diced preserved cadaver cartilage grafts rather than autogenous cartilage grafts may be employed as a substitute for metallic foreign-body implants when the prognosis is doubtful.

Other Uses in Facial Region

Diced-cartilage grafts have also been used to repair mastoid fistula, to build out the prominence of the malar bone, to correct receding chin, and, indeed, to restore contour in any bony deficiency of the face as a substitute for bone grafts.

Grafts of this type packed in a perforated vitallium mold will conform to the shape of the mold like wet sand in a sand mold (21). When a perforated ear mold is filled with cartilage segments and buried beneath the patient's abdominal skin, connective tissue and blood vessels grow through the openings in the mold. *In situ* they nourish the cartilage and fasten the separate segments together in a solid plaque. If the mold remains in place for about five months, the plaque will be bound together by mature connective tissue with tough collagenous fibers, so that the cartilage can be removed from the mold as a solid ear structure. This cartilage framework may be inserted beneath the skin in the region of an absent

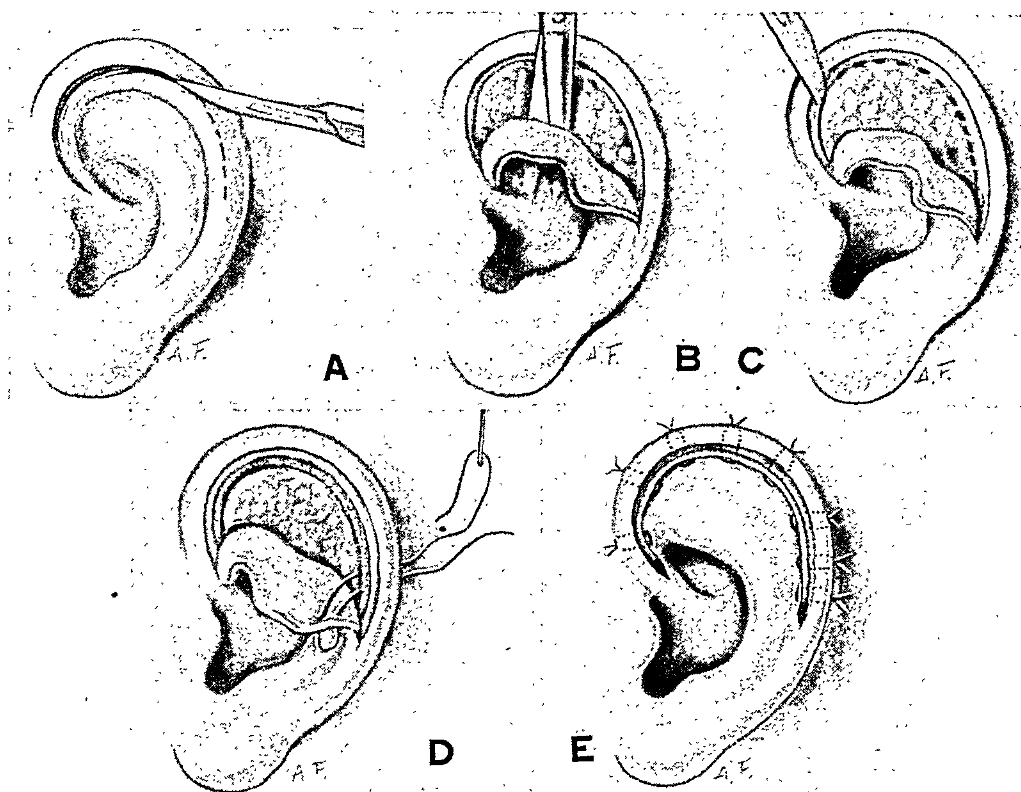


Fig. 52. Final trimming operation to sharpen helix, increase depth of concha and canal region and remove hair bearing skin. (May be done in one or two stages depending on abundance of circulation in skin)

The contours of the reconstructed ear will not be as distinct as is indicated on this drawing due to the thickness of the covering skin, the formation of fibrous tissue beneath the skin and compression of the cartilage against the rigid skull bone.

A. An incision is made through the skin covering of the helix down to the diced-cartilage framework of the ear. This incision should reduce the width of the helix by about ½. Most of the hair bearing skin will be located below this incision.

B. The skin is dissected from the underlying diced-cartilage framework over the scapha and well down into the depth of the conchae.

C. An incision is made deeply into the cartilage and sufficient cartilage is removed to reduce the width of the helix and increase its prominence. It may be necessary to carry this incision down to the skin covering on the posterior surface of the auricle and remove a solid wedge of cartilage. This will allow the cartilage rim of the helix to roll downward producing a very normal affect.

Cartilage and fibrous tissue are widely removed in the region of the conchae leaving prominent ridges to accentuate the anterior and inferior crura. It may be necessary to remove all cartilage in the depth of the conchae.

D. The dissected flap of skin will be larger than is required to snugly cover the raw defect. This permits excision of hair bearing skin on the upper portion of the skin flap.

Mattress sutures are inserted through the posterior surface of the auricle to draw the free margin of the skin flap into the cartilage groove or into actual contact with the deep surface of skin lining the back of the auricle.

E. Mattress sutures tied on posterior surface of auricle. The upper free margin of skin is not sutured because it tends to give a very natural appearance to the helix when allowed to heal without suture.

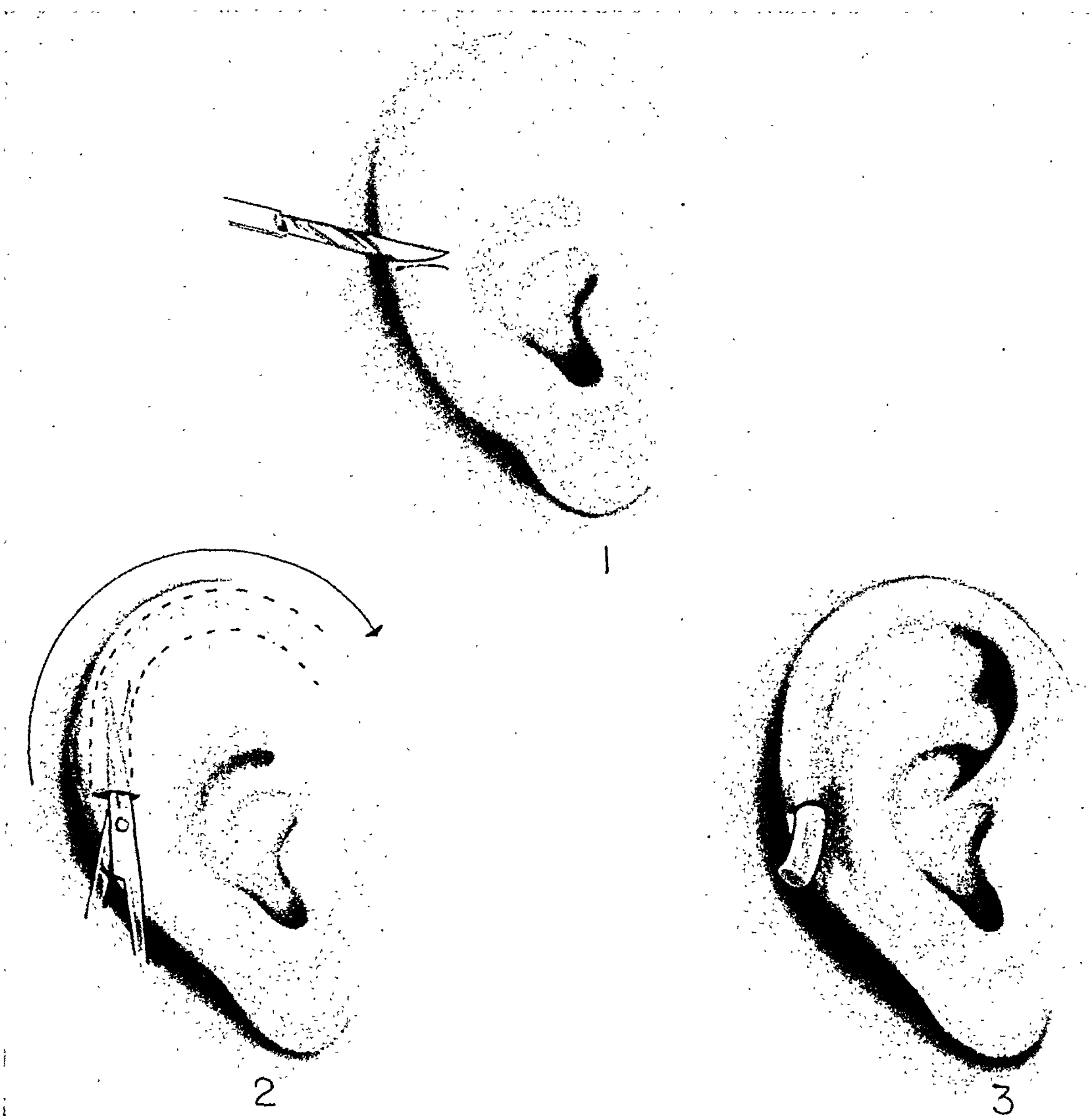


FIG. 53. Introducing a curved rib cartilage graft to simulate the helix of the reconstructed ear.

ear to form the structural support for a new auricle.

It is interesting to note that the cartilage plaque is entirely autogenous, being composed of the patient's own cartilage, which is bound together by autogenous connective tissue and supplied by autogenous blood vessels. The cells and matrix of the cartilage, the connective tissue, and many of the blood vessels survive this free transplantation, and a new blood-vessel supply arises mainly through end-to-end anatomosis between severed blood vessels in the host bed and those in the graft. Some new penetrating capillary growth occurs from the host vessels into the substance of the connective tissue of the graft. Probably this new penetrating capillary growth later connects with the surviving vascular system of the graft.

Diced-Cartilage Grafts in Other Surgical Fields

The extreme malleability of diced-cartilage grafts and the conformity of small segments to a perforated mold of any

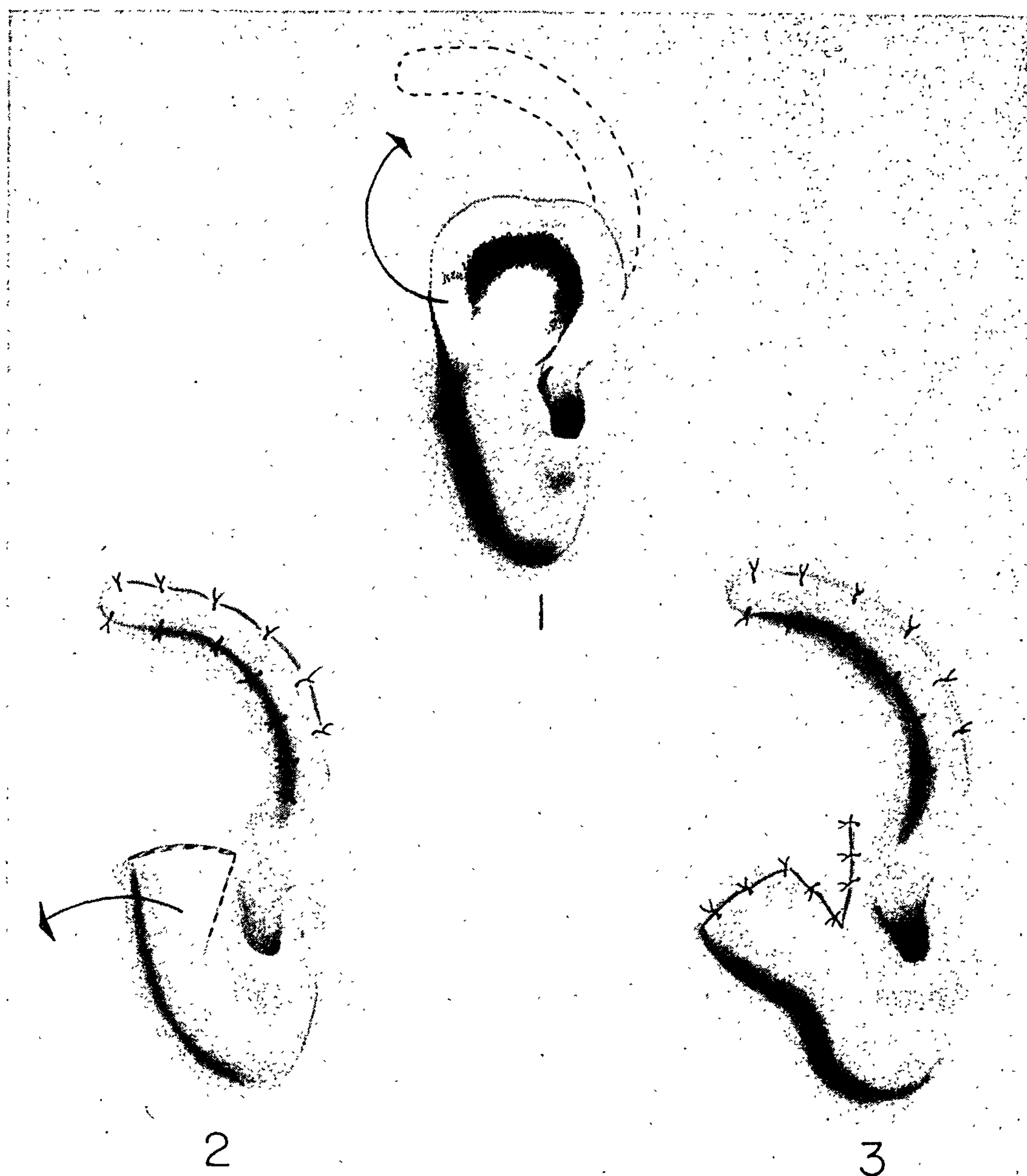


FIG. 54. Demonstrating the principle of using all portions of the auricle which are present in partial ear reconstruction. Diced-cartilage grafts will later be introduced to provide support where the patient's cartilage is deficient. Sometimes an ear mold is used and the parts of the diced cartilage ear not required are cut away.

desired shape led me to suggest their use to a number of surgical colleagues at St. Barnabas Hospital.

Dr. John Flanagan of Newark in collaboration with the author became interested in the idea and designed a perforated vitallium mold in the shape of a thin concave-convex disc which might be employed on the principle of the ear mold to form a new articular surface of cartilage. If inserted over the head of the femur, the cartilage disc might permit movement in ankylosis of the hip joint. The vitallium mold[4] was filled with diced segments of a patient's rib cartilage and buried

[4] Made by the Austenal Company of New York City.



FIG. 55. Lowering the hair-bearing scalp skin to replace the upper part of the hairless split skin graft is often the final step in total ear reconstruction.

beneath the abdominal skin. When the mold was removed after a period of four months, the cartilage segments were firmly bound together in the form of a thin cartilage cap. With the cap inserted as a cover for the head of the femur, the patient at the present time—two years after the operation—is walking and weight-bearing about as well as if a vitallium cap had been used.

Dr. George Simms of Rutherford, New Jersey, has successfully used diced cartilage to reinforce the fascia in recurrent ventral and inguinal hernia. One of his patients with a large ventral herniation had a recurrence of the hernia following operative repair with a dermagraft. At a second operation diced cadaver cartilage grafts from the cartilage bank were introduced over the surface of the thin muscle fascia and in some areas directly over the peritoneum. The subcutaneous tissue and abdominal skin were then sutured over the diced-cartilage grafts and a firm pressure dressing applied. The diced-cartilage grafts became bound together by connective tissue in the form of a large pancake-like

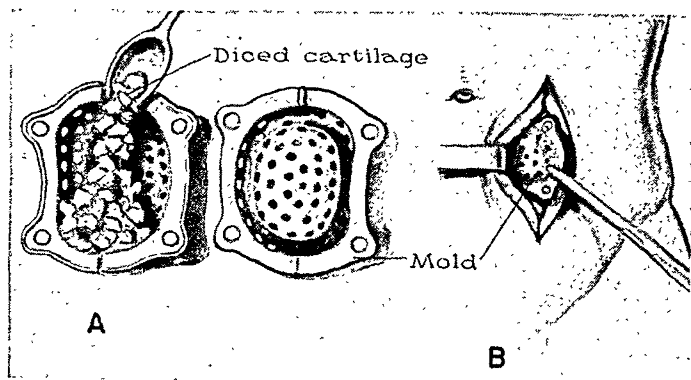


FIG. 56. Diced cartilage grafts used to form a cartilage cover for the head of the femur in ankylosis of the hip joint.

A. Perforated vitallium mold (made by Austenal Company, New York, N. Y.), is filled with autogenous diced-cartilage grafts following the ear mold principle.

B. Mold with its cartilage filler introduced beneath patient's abdominal skin and fatty layer.

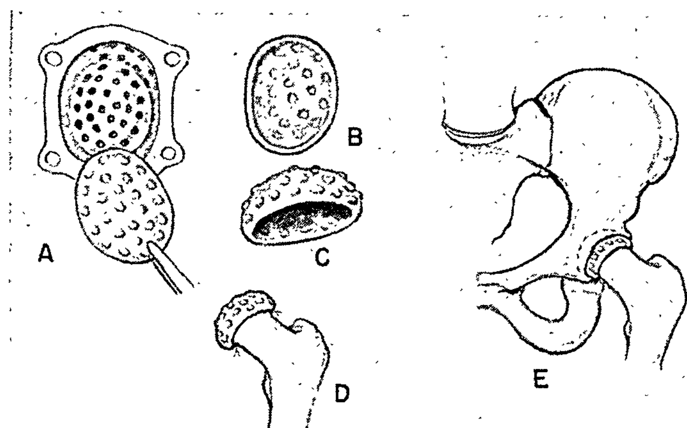


FIG. 57. A. Mold removed after 5 months and cartilage cap removed.

B and C. Cartilage cap composed of diced-cartilage grafts bound together by connective tissue in the form of a solid structure.

D. Diced cartilage cap covering freed head of femur.

E. Head of femur with cartilage cover inserted in acetabulum.

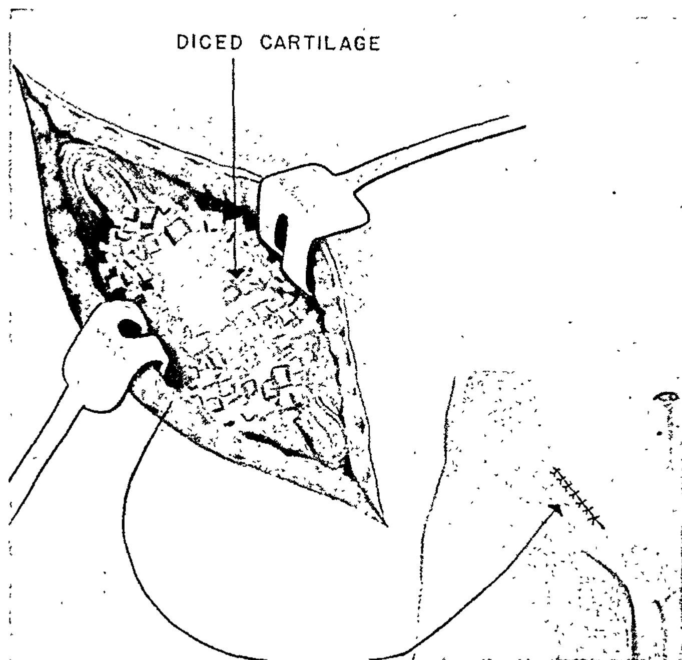


FIG. 58. Diced-cartilage grafts used to reinforce deficient fascia in recurrent inguinal hernia.

plaque, which served as an internal or buried truss. The patient has no recurrence of this large ventral hernia two years after operation.

With the assistance of the author, Dr. Henry Brodkin (22) of Newark has built up several new ribs to protect the heart in a patient who had had extensive removal of

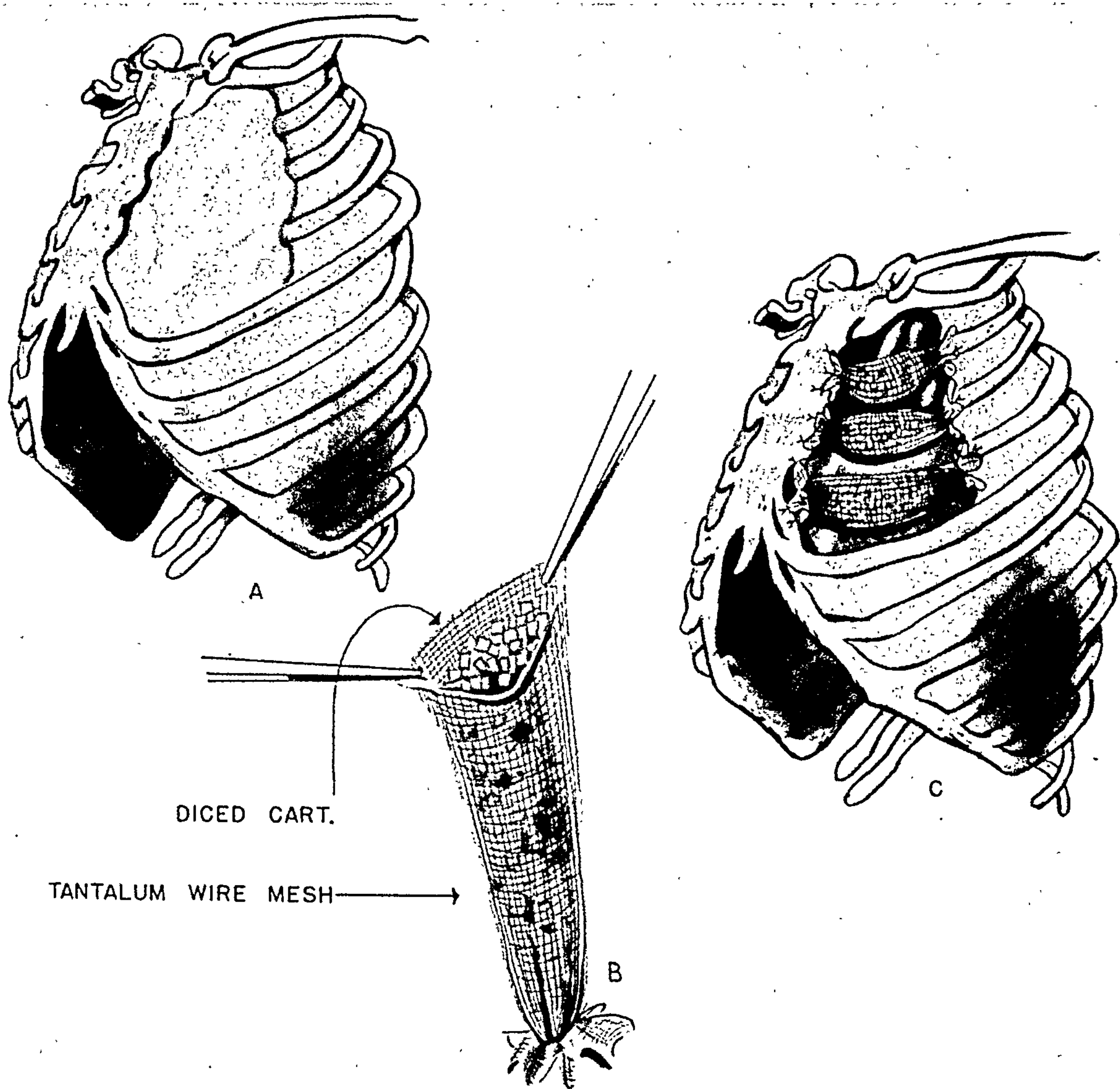


Fig. 59. Diced-cartilage grafts used to form new ribs for the chest cage.

the cartilage. This was accomplished by introducing diced cadaver cartilage grafts in tantalum mesh and fastening the open ends of the mesh with a purse-string wire suture to retain the mass of cartilage segments within the mesh. The tantalum mesh with its content of cartilage was sutured to the sternum at one end and the posterior rib segments horizontally at the other end or, in some instances, in a vertical direction so as to bridge the defect in the chest cage. Connective tissue grew through the openings in the mesh and bound the cartilage segments together in the form of a solid rib structure, which served to give support for the soft tissues of the chest and to provide protection for the heart. Diced-cartilage grafts have also been used to restore normal contour in funnel-chest deformities.

In collaboration with Dr. Robert Green of South Orange, New Jersey, and with Dr. Richard Swain of Newark, diced cartilage has been used for filling large bony defects in infants with spina bifida. The method is indicated particularly in patients with a large cord herniation, associated with an extensive bony defect and insufficient fascia, muscle, and skin for adequate closure and coverage. Double pedicled skin flaps are elevated at the sides and resutured in place

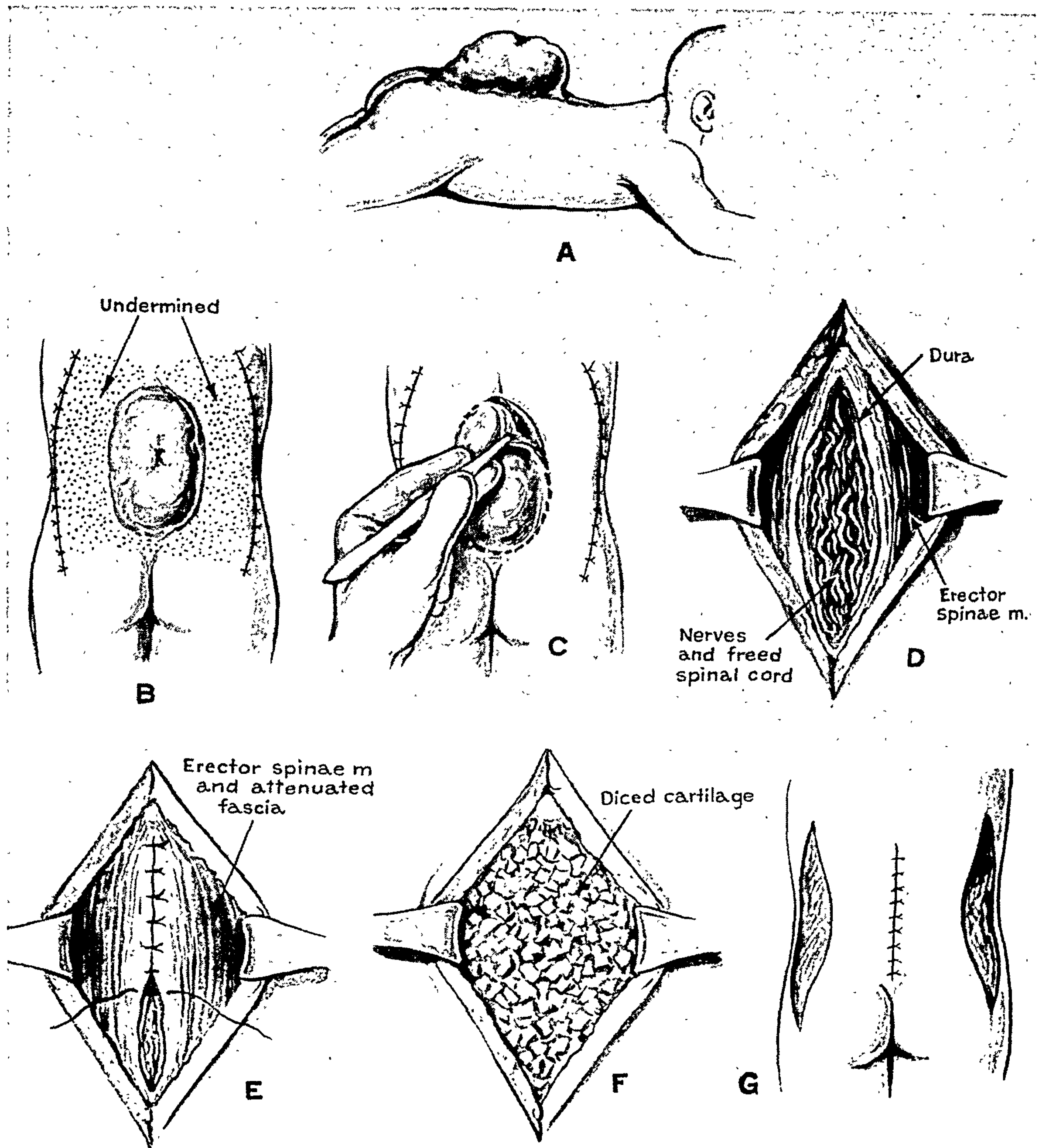


FIG. 60. Use of diced-cartilage grafts for spina bifida with large bony defect in spinal canal.

A. Infant with large spinal canal defect and herniation.
B. Vertical skin flaps delayed to insure adequate blood supply.
C. Thin epidermis covering herniation excised and spinal fluid drained out.
D. Cord elements placed in spinal canal.
E. Idealized drawing showing complete approximation of fascia; this is seldom possible in extensive defects.
F. Diced-cartilage grafts (bank cartilage) introduced to cover area of spinal canal defect.
G. Skin flaps sutured over plaque of diced-cartilage grafts.

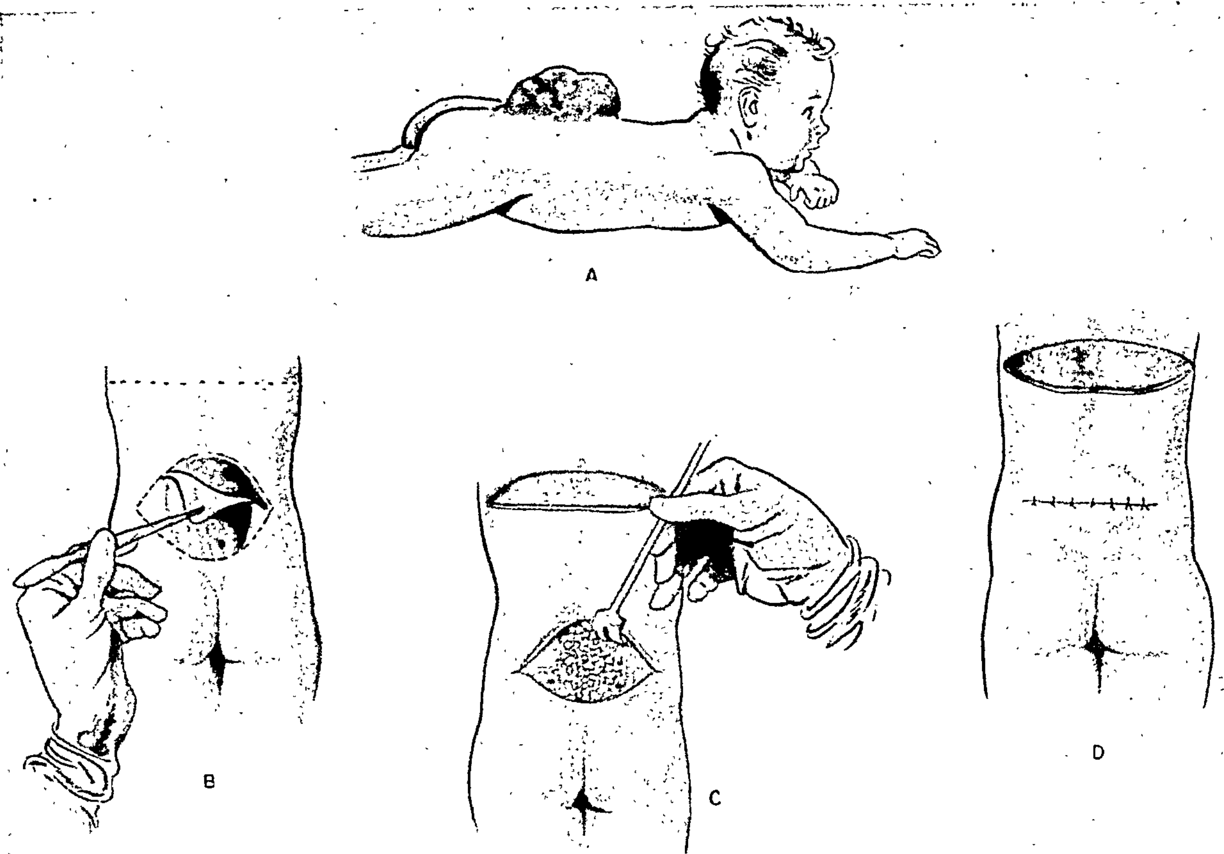


FIG. 61. Use of a single horizontal bipedicled skin flap to cover defect in spina bifida.

to allow adequate blood supply to develop in the flaps. Two weeks later the thin skin covering the herniation is removed, the cord elements reduced and the best possible closure obtained by suturing fascia and muscle over the bony defect. Diced cadaver cartilage grafts are then introduced over the fascia and muscle, and sometimes directly over the cord content. A sheet of tantalum or stainless-steel screen may be sutured over the defect to keep the diced-cartilage grafts out of the spinal cord opening when fascia and muscle closure is inadequate. The lateral skin flaps are then elevated and sutured to cover the plaque of the graft. If the child does not die from hydrocephalus or meningitis, the diced-cartilage grafts will become bound together by fibrous tissue and the solid plaque will prevent a herniation of nerve elements from the spinal cord.

Discussion

It appears that diced-cartilage grafts may have a much wider field of application than was originally supposed. The author first designed and employed them to fill skull depressions and defects about the face. Later on the grafts were packed in a perforated ear mold, and when this mold with its cartilage content was transplanted into human abdominal fat the separate segments became bound together by ingrowing fibrous tissue so that a solid structure was formed which accurately represented the shape of the mold.

The method of simply introducing the cartilage grafts to fill a cavity or to prevent herniation as in skull depressions, inguinal and ventral hernia, and spina bifida, and the method of inserting the grafts in a special mold or form as in ear reconstruction, ankylosis of joints and the formation of new ribs *are all based on a physiological fact or*

law. Connective tissue seems to abhor unlined and artificial dead spaces as nature abhors a vacuum. There are numerous dead spaces between the individual diced-cartilage segments; and fibroblasts, with their accompanying blood vessels, will always grow into these spaces and fill them with collagenous fibers and cement substance. This collagenous cement when dry (after about five months) provides a strong binding material that holds the individual cartilage segments firmly together.

The simple procedure of introducing living or dead cartilage grafts in a cavity or the method of packing them in a mold may be selectively employed in different surgical fields for various purposes.

It is fortunate that cadaver bank cartilage grafts are usually well tolerated by human tissues. This allows the surgeon to provide support for extensive defects in the chest wall, for herniation of both sides of the abdomen, and for extensive spinal and other skeletal deficiencies in infants and young children when it is not wise to use the patient's own cartilage.

One should bear in mind, however, that autogenous tissues, as free grafts, are always better than the tissues of another individual. Obviously, cadaver cartilage must be used when the patient's own cartilage cannot be obtained in sufficient quantity.

SUMMARY COMMENT

Autogenous cartilage is always the tissue of choice for grafting purposes. Preserved or fresh homogenous cartilage grafts are a valuable second choice, and heterogenous cartilage from the ox, sting ray or shark should be considered only when homogenous cartilage is not available, if it should be considered at all.

The reason for the long survival time of the cells in fresh homogenous cartilage grafts is quite possibly the protective nature of the gel-like matrix, which prevents the entry of host antibodies. A similar substance is present in cornea and lens (8), and in the capsules of pneumococci (23).

In humans there is good evidence that young cartilage grafts do not grow or increase in size after transplantation, regardless of the presence or absence of perichondrium.

Attention is called to the advantages of introducing a cartilage strut through an incision in the buccal mucous membrane for support of the nasal tip in saddle nose deformity. This strut, which can also be used to correct retraction of the columella, should be located in front of the beam of cartilage supporting the dorsal line of the nose.

Diced-cartilage grafts have a wide clinical field of application. Their successful use is dependent upon a simple physiological theory or law: *Connective-tissue cells appear to abhor unlined and artificial dead spaces in the body as nature abhors a vacuum.* The numerous dead spaces between the diced-cartilage segments are occupied by fibroblasts, and these fibroblasts elaborate collagenous fibers and cement substance which serve to bind the cartilage grafts together in the form of a solid plaque.

The clinical use of ox cartilage by Gillies and others is not criticized. Gillies has forced plastic surgeons to consider the merits of the cartilage heterograft and to produce evidence against its use. One should not ignore the possibility that future discoveries may reveal some way to render the cartilage heterograft more acceptable to human tissues.

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PART III

Bone

CHAPTER 15

Structure of Bone

Bone has a relatively simple structure for, like cartilage, dense fascia and tendon, it is composed of only one type of parenchymal cell surrounded by a large amount of non-living intercellular material or matrix. The inanimate intercellular material, which was the product of activity of the bone cells (or of some cells), is rigid due to a calcified substance.

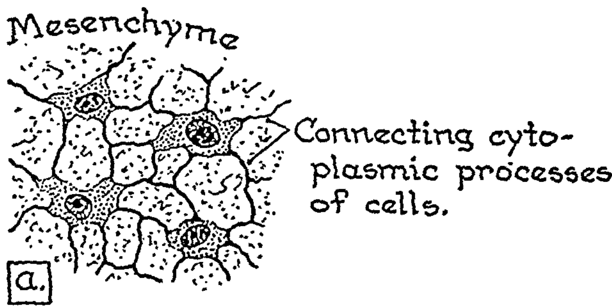
Bone is derived from mesoderm, and consequently its parenchymal cells are descendants of primitive mesenchymal cells which have become specialized as bone cells instead of cartilage, fascia or tendon cells, all of which arise from mesenchyme.

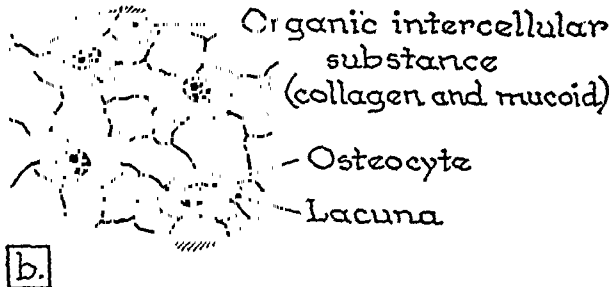
Ham (1) has suggested that nature, in an attempt to improve the weight-bearing ability of hyaline cartilage, tried the expedient of impregnating its intercellular substance with mineral salts. The experiment was not successful, however, because calcium deposits interfere with the diffusion of waste products, food substances and gases throughout the intercellular substance of cartilage. Hence the cells, deprived of their means of existence, die. Thus, calcified cartilage soon becomes dead cartilage, and for some unknown reason dead cartilage tends to be absorbed and eventually disappears.[1] According to Ham, bone was evolved to overcome this peculiar deficiency of calcified cartilage.

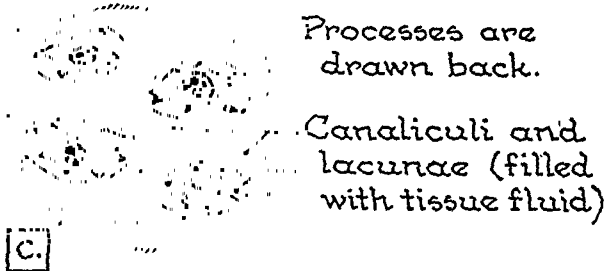
Bone resembles cartilage since it consists of cells in lacunae surrounded by relatively large amounts of dead intercellular substance. This intercellular substance is similar to the matrix of cartilage, with the important exception that it contains mineral salts which give the tissue additional strength but necessitate an entirely different circulatory mechanism to maintain the bone cells.

The matrix of bone, therefore, unlike cartilage, contains a system of small channels called *canaliculi*. These channels connect the lacunal chambers, with their bone cells, to one another and extend to haversian canal areas in the bone where capillaries are present. In this way tissue fluid from the capillaries can permeate through the tubular canaliculi to all lacunal chambers and supply the needs of the individual bone cells. Thus, the bone cells remain alive despite the calcified nature of their rigid intercellular substance.

[1] Certain large aquatic forms such as the shark and giant sting ray have retained their skeletons in the form of hyaline cartilage. This they could do, since their body structure tends to be buoyed up by the liquid medium in which they live. In older sharks the cartilaginous skeleton tends to become ossified, but this is not followed by bone replacement according to most authorities.







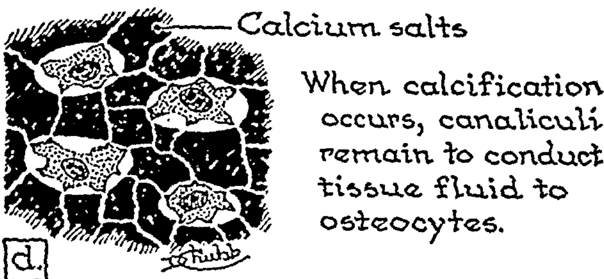


FIG. 62. Diagrams to show how bone develops and how the cytoplasmic processes of the cells account for canaliculi, which thereafter provide a means for the cells to be nourished even though the organic intercellular substance about them becomes calcified. From Histology, Arthur Worth Ham. Philadelphia: J. B. Lippincott Co., 1950.

DEVELOPMENT OF BONE

It is important to know that *cartilage is not changed into bone.*[2] One might suppose that it would be simpler if this were so, but nature has determined an entirely different method for bone formation. In the development of the body, temporary cartilage models of the future bones are first formed in the embryo. Parts of these become calcified and bone formation takes place as the calcified cartilage is absorbed piece by piece. *In this way bone formation replaces calcified cartilage; calcified cartilage is not utilized as part of the new bone structure.*

Earlier physiologists had believed that the transformation was direct, the fundamental cartilaginous substance becoming, through deposition of calcium salts, the fundamental bone substance, and the cartilage cells becoming bone cells. Johannes Müller (3) demonstrated in 1836 that cartilage does not become bone directly but undergoes absorption and is replaced by embryonic connective tissue, which becomes ossified.

In a second type of bone formation, which was unknown for a longer time, *there is a direct transformation of connective tissue into bone without the intervention of cartilage models.* This type, described by Heinrich Müller (4), Virchow (5), Sharpey (6) and others, gives rise to the *membranous bones.*

It is possible that morphologists have overemphasized the difference in origin of the cartilaginous and membranous bones of the human body. Actually the origins are quite similar, as both types develop by transformation of embryonic or adult connective tissue into a calcified connective

[2] Cartilaginous tissue may become bone without substitution and without previous destruction. This chondro-osseous metaplasia is infrequent in the normal evolution of human organisms but has been noted in the course of pathological osteogenesis and in the bone development of lower vertebrates. (Leriche and Policard's "*The Normal and Pathological Physiology of Bone*" (2). This volume contains more stimulating speculation and careful analytical conclusions than any other work on bone grafting known to the author. The two translators, Dr. Sherwood Moore and Dr. J. Albert Key, are to be congratulated on their able work.)

tissue. Leriche and Policard (7) have aptly remarked that "experimenters have been hypnotized in their research by the part played by cartilage in bone formation instead of the vital rôle of the connective tissue; they have in fact chosen the poorer substance for study."

The generally accepted belief, currently expressed by histologists, is as follows: Some of the mesenchymal cells in the area where bone is to be produced differentiate into osteoblasts, which secrete or otherwise form the characteristic organic intercellular substance of bone (8).

Stearns (9) demonstrated that collagenic fibers are produced by a budding off of substance from the cytoplasm of fibroblasts in the process of wound healing. One may assume therefore that the collagenic fibers in bone are formed in a similar way. The inorganic material, which consists mainly of calcium phosphate, is deposited in and impregnates the fibrous structure. It appears that there is a continuous physiological turnover of the inorganic salt contents of bony tissue throughout life. These salts are employed as a reserve to be drawn upon to meet metabolic requirements elsewhere in the body and they are continuously being replaced by the deposition of new material (10).

Osteoblasts

Many of the osteoblasts are surrounded by their own secretion and become the smaller osteocytes or bone cells in the newly-formed bone. This process of metaplasia is essentially the same in the formation of both cartilaginous and membranous bones.

Wherever bone forms, special bone-building cells, the osteoblasts, must be present as these cells alone have the capacity to form bone.[3]

The osteoblasts, which are destined to become bone cells, have numerous cytoplasmic processes which extend from cell to cell and to the neighboring small blood vessels. These are withdrawn when the intercellular substance sets about them, thus giving rise to the canaliculi or small canals in bone.

Osteoblasts on the surface of developing bones, whether of the skull, face, or long bone variety, continue to proliferate, and some of the offspring are surrounded by calcified matrix, in this manner becoming bone cells. This new appositional bone is usually deposited in the form of rather even layers, called *lamellae*.

Osteoclasts

It is obvious that on some surfaces of developing bones absorption must take place along with new bone formation, so that normal contour and function are provided for them as supportive and protective structures. The exact mechanism by which absorption of bone takes place is not definitely known. In areas where absorption is occurring, however, multinucleated giant cells, called *osteoclasts*, are frequently seen in small concavities termed *Howship's lacunae*.

Formerly these osteoclasts were regarded as cells with the specific function of bone resorption. As knowledge increased, however, it became apparent that the problem of bone resorption was somewhat more complex than was first supposed, and it now seems unlikely that the resorption of bone is accomplished only by specific cellular activity (11). According to Ham, the problem of bone resorption is best viewed by considering it as a *failure in bone maintenance*. The evidence suggests that bone, at any surface not protected by a covering of cells that manufacture phosphatase, tends to dissolve away.

[3] Leriche and Policard disagreed with the classical theory regarding the active bone-forming capacity of osteoblasts. They noted bone formation in the complete absence of osteoblasts.

Growth of Bone

By processes of appositional deposit, resorption, growth in length of long bones, and growth at the suture lines in other bones such as those in the skull and palate, the bones are remodeled for some time after birth to provide for the needs of the developing human. Cowdry (12) notes the orderliness of normal bone development. Each kind of bone grows according to its own schedule, which involves a delicately-regulated balance between bone formation and bone resorption, between simultaneous deposition of calcium in some parts and removal of calcium from others. Bone as a tissue is particularly affected by functional use or lack of use. Use leads to strengthening and disuse to atrophy.

It is important to note that bone can grow only by an appositional mechanism. The intercellular substance of bone becomes calcified as soon as it is formed, and it is not sufficiently malleable to expand and make room for dividing bone cells within the substance of bone. On the other hand, cartilage is present in the body normally in an uncalcified condition, and in this state its matrix is sufficiently malleable so that division of cartilage cells with interstitial growth can and does occur.

Intercellular Substance

The apparently homogeneous intercellular substance of fresh bone contains masked osteocollagenous fibers similar to the collagenous fibers of loose connective tissue and to the masked fibers in hyaline cartilage. By special staining methods it can be shown that the individual fibers are often connected in small bundles which are united by an amorphous binding substance (13). It is in this organic amorphous binding substance that the inorganic mineral constituents of bone are laid down.

The organic portion of compact bone is made up chiefly of bone collagen or ossein; only a small fraction of the dry weight is contributed by the bone cells. The organic framework yields gelatin when boiled (14).

The mineral content in mature bone is about 80 per cent calcium phosphate, 13 per cent calcium carbonate and 2 per cent magnesium phosphate, with smaller amounts of other substances (15). The composition does not exist as a mixture of these salts, however, but as a complex salt probably of the apatite series in the form of submicroscopic crystals.

ARCHITECTURE OF BONE AND NUTRITION

Almost all bones are formed of two types of tissue. The hard cortex of a bone is dense compact tissue with scanty blood vessels, whereas the cancellous or spongy bone located in the medulla has a porous or open structure and a rich vascular supply.

Cancellous bone consists of a network of plates and bars located and arranged in directions that correspond with lines of tension or stress to which the particular bone is subjected. The trabeculae of cancellous bone are composed of a number of adjoining bone plates or lamellae. The osteocytes are located in lacunae within the substance of the lamellae and these spaces communicate with each other through a network of canaliculi.

In compact cortical bone the lamellae are arranged regularly in a manner closely related to the distribution of the blood vessels in the haversian canals, which provide nourishment for the bone cells (16).

An individual haversian canal contains one or more blood vessels, which are usually capillaries and venules in close association with loose connective tissue that fills the remainder of the space. For all practical purposes these haversian canals are the only openings from the medullary cavity or the outer surface of the bone into the hard substance of the cortex. Blood vessels in

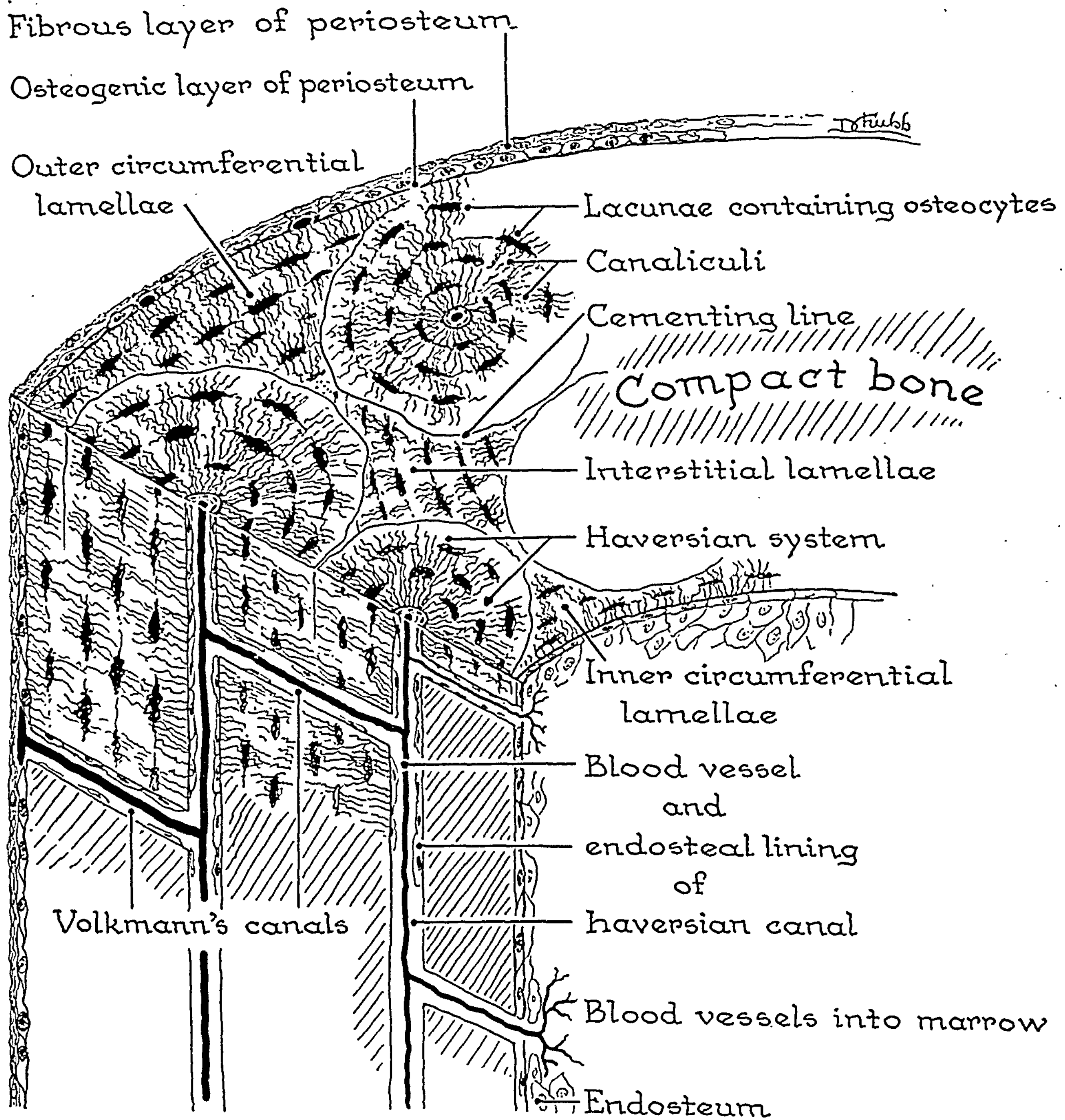


Fig. 63. A three-dimensional diagram showing the appearance of both a cross section and a longitudinal section of the shaft of a mature long bone. This diagram shows the relationship between the blood vessels of the periosteum, Volkmann's canals, haversian canals and the marrow cavity. From Histology, Arthur Worth Ham. Philadelphia: J. B. Lippincott Co., 1950.

the haversian canals provide nourishment and remove waste products from the cell in a large number of different haversian systems, each of which is a *closed system* that does not ordinarily communicate with adjacent ones. Thus, if the cells in a free graft of cortex bone are to survive transplantation, the host tissue must provide blood vessels to establish a rather quick anastomosis with arterial and venous

channels in the haversian canals of the graft to nourish the cells in the separate haversian systems.

The canaliculi of the haversian systems are extravascular, their function being to promote the diffusion of tissue fluids required for the maintenance of the osteocytes and the interstitial substance of the bone. The earlier histologists were so impressed by the easily demonstrable canaliculi in bone that they hypothesized and even described tiny channels of a similar nature in cartilage. It is now known that the channels described in cartilage were artifacts and that there are no demonstrable channels between blood vessels in the perichondrium and the cartilage cells.

Periosteum

In the literature dealing with bone considerable disagreement is evident regarding the exact composition of periosteum and its physiological use. There is no doubt whatever concerning the importance of the blood supply which it provides for the outer cortical layer of a long bone and often for the complete outer cortex of the skull. This is known to all surgeons, because nutritional death and sequestration of the outer cortical layer of bone are often observed when the periosteum is stripped away and the bone exposed. Some aspects of the osteogenic or bone-forming capacity of the periosteum, however, have given rise to divergent views, although this premise is accepted by most of the present-day histologists.

Ollier (17) believed that the inner layer of periosteum intimately in contact with bone was the true osteogenic or cambium layer. He performed numerous experiments on animals in which he elevated strips of periosteum with a sharp elevator and after transplanting them into soft tissues, observed that bone formation occurred in the periosteal transplant. It is important to note, however, that Ollier in his anxiety to include the inner layer of periosteum with his transplant often detached small bits of bone which were transplanted with the periosteum.

Leriche and Policard, who dedicated their book on bone physiology to Ollier and admired him greatly, failed to verify his findings regarding the osteogenic capacity of periosteum. These two authors, working in collaboration, were not able to produce bone from periosteal transplants in animals or in man excepting when particles of bone were included with the periosteal transplant (18).

Leriche and Policard were brought up in the tradition of their famous fellow countryman, Ollier, and had been taught to consider his teachings as one of the most firmly grounded in surgical pathology. Their first researches were inspired by the classical teachings and established more firmly the technical value of the principles that Ollier stated. As soon as they met with the details of the phenomenon it seemed evident that, if the facts brought forward by Ollier were absolutely unassailable, the interpretation which he had given them was to be rejected entirely. Ollier was not a histologist. In his time histologists were rare. He seldom used the microscope and never at first hand.[4]

After extensive experimental work Leriche and Policard concluded that the inner osteogenetic or cambium layer of the periosteum *does not exist.*

Macewen (19) also was unable to verify Ollier's experimental work concerning the osteogenic power of transplanted periosteum. He decided that the periosteum is not osteogenic but, instead, is only a limiting membrane that surrounds the shaft of a bone.

[4] The writings of Ollier, Macewen, Leriche and Policard, and Albee, among others, are well worth reading in the original and should be in the library of every one interested in bone grafts. Curiously, a great number of recent articles do not have the value or stimulation of these older ones.

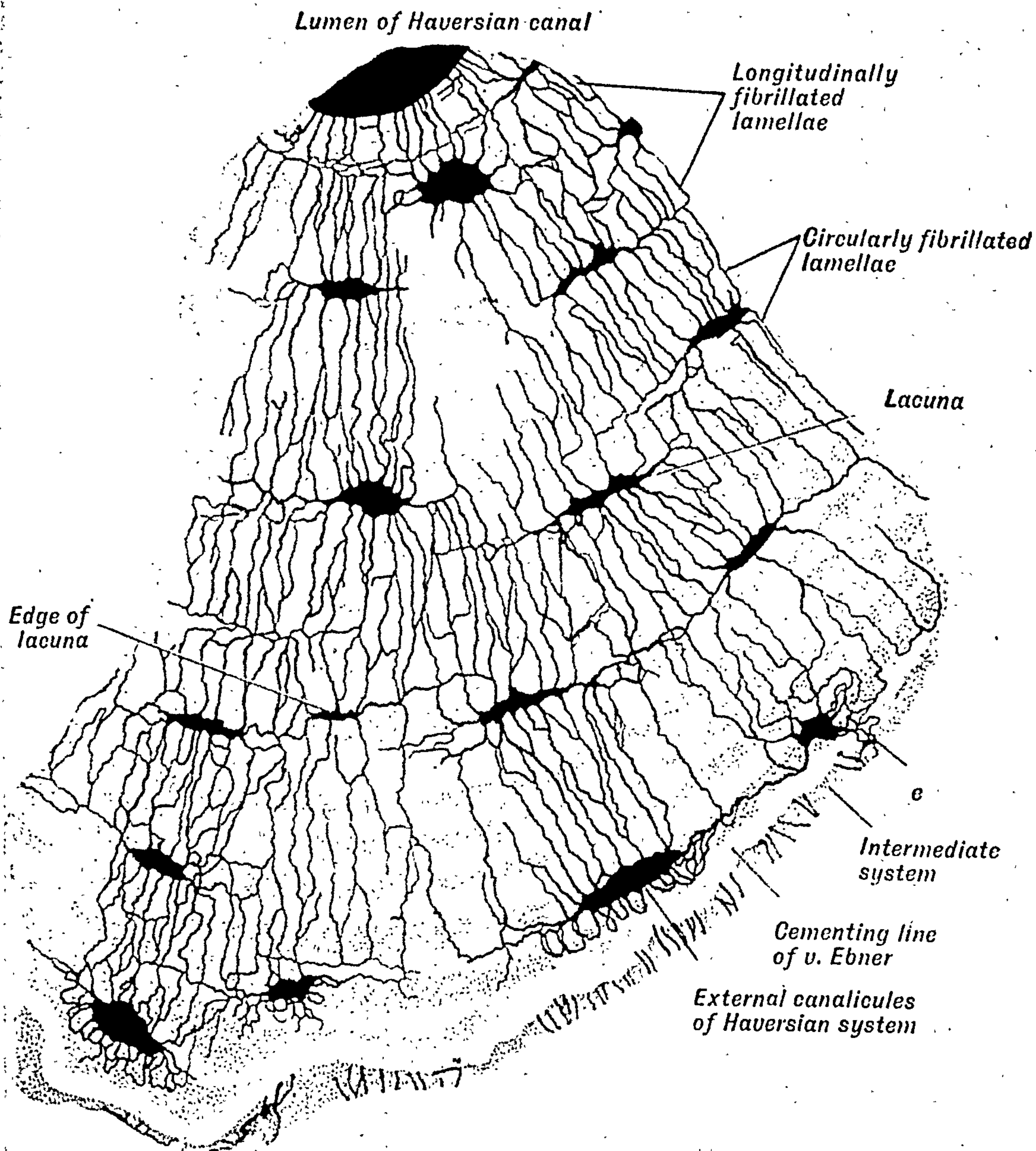


FIG. 64. Sector of a cross section of an haversian system of a macerated human hip bone. The cavities and canalicules are filled with a dye: *e*. connection of canalicules of the haversian system with those of an intermediate system. 520×. (A.A.M.) From A Textbook of Histology, 5th ed., Alexander A. Maximow and William Bloom. Philadelphia & London: W. B. Saunders Co., 1948.

Present-day histologists tend to consider periosteum as consisting of two layers—an outer fibrous one that is made up of collagenic fibers and fibroblasts, and an inner osteogenic layer that consists of osteogenic cells or osteoblasts. This viewpoint is in accord with that of Ollier, who believed that the inner periosteal layer was essential for the successful survival of bone grafts.

I buried two strips of perichondrium from the rib in the chest fat as human autografts; bone formation failed to occur. Human autografts of rib, tibial and iliac bone with periosteum on the outer surface were transplanted into abdominal fat. These grafts became progressively smaller and could not be identified twelve months after transplantation. Ollier observed that transplanted

perichondrium, including the inner osteogenic layer (cambium layer) and some attached bone, gives rise to new bone formation in animals. In humans, however, this does not seem to occur, or at any rate it does not consistently occur. Animal experimental work is suggestive but *never conclusive* in regard to the behavior of tissue grafts in the human. Fresh autogenous and even homogenous cartilage grow in the rabbit; they do not grow in the human. The epidermis of buried free skin grafts persists and produces *epithelial-lined cysts in dogs; in humans the* epithelial layer is usually absorbed and cyst formation rarely occurs.

Endosteum

This tissue is the thin membrane that lines the walls of the bone cavities—which usually contain bone marrow—and extends into the haversian canals. It consists of a single layer of cells which as a rule are a mixture of osteoclasts and osteogenic cells (osteoblasts). It is believed by some that the endosteum has osteogenic powers and aids the periosteum in the process of new bone formation following fracture.

OSTEOGENESIS

Ossification and true bone formation may occur in tissues quite unrelated to bone, as in muscles, in the walls of arteries or in the fibrous tissue of old scars. It appears that certain connective-tissue elements all over the body have the ability to form new bone. Epithelium of the urinary tract, if transplanted into a sheet of fascia in any part of the body in animals, becomes surrounded by bony tissue (20).

I have noted bone formation many times outside and within the substance of both living autogenous cartilage grafts and preserved homogenous cartilage grafts buried in human fat. Curiously, new bone formation has not been seen in humans around living or preserved bone grafts buried in fat. When such grafts are from the ribs, ilium, or tibia they are absorbed in about the period of a year regardless of the presence or absence of periosteum on the grafts. Fresh autogenous bone grafts from the nasal septum, nasal bones, and turbinates transplanted without periosteum retain their bony structure after burial in abdominal fat. These grafts become denser in structure but new bone formation does not occur in or about the grafts.

Osteoblastic Theory

The *osteoblastic theory ascribing osteogenesis* to special cells in the periosteum is accepted by most histologists today. One feels, however, that they are taking the easy way out in an understandable effort to instruct undergraduate students. Certainly osteogenesis or new bone formation has been observed in humans when there is a complete absence of periosteum. An example of this finding is the new bone formation occurring in preserved dead homogenous cartilage grafts buried in abdominal fat and in fresh autogenous cartilage grafts buried in fat.

If it is possible that osteoblasts in the periosteum have the ability to produce new bone or to initiate and supervise the process of osteogenesis, it is certainly probable that undifferentiated connective-tissue cells in fat, muscle and other tissues also have this same ability. This latter method of bone formation, advanced by Leriche and Policard, is called the *mesenchymal theory* in contradistinction to the *osteoblastic theory* advanced by Ollier.

Mesenchymal Theory

Leriche and Policard (21), in an article in 1926 and in their book (22) published in 1928, concluded that osteoblasts do not have an active role in bone formation. Instead, bone formation is the result of a metaplastic change in connective tissue and may occur anywhere in the body regardless of the pres-

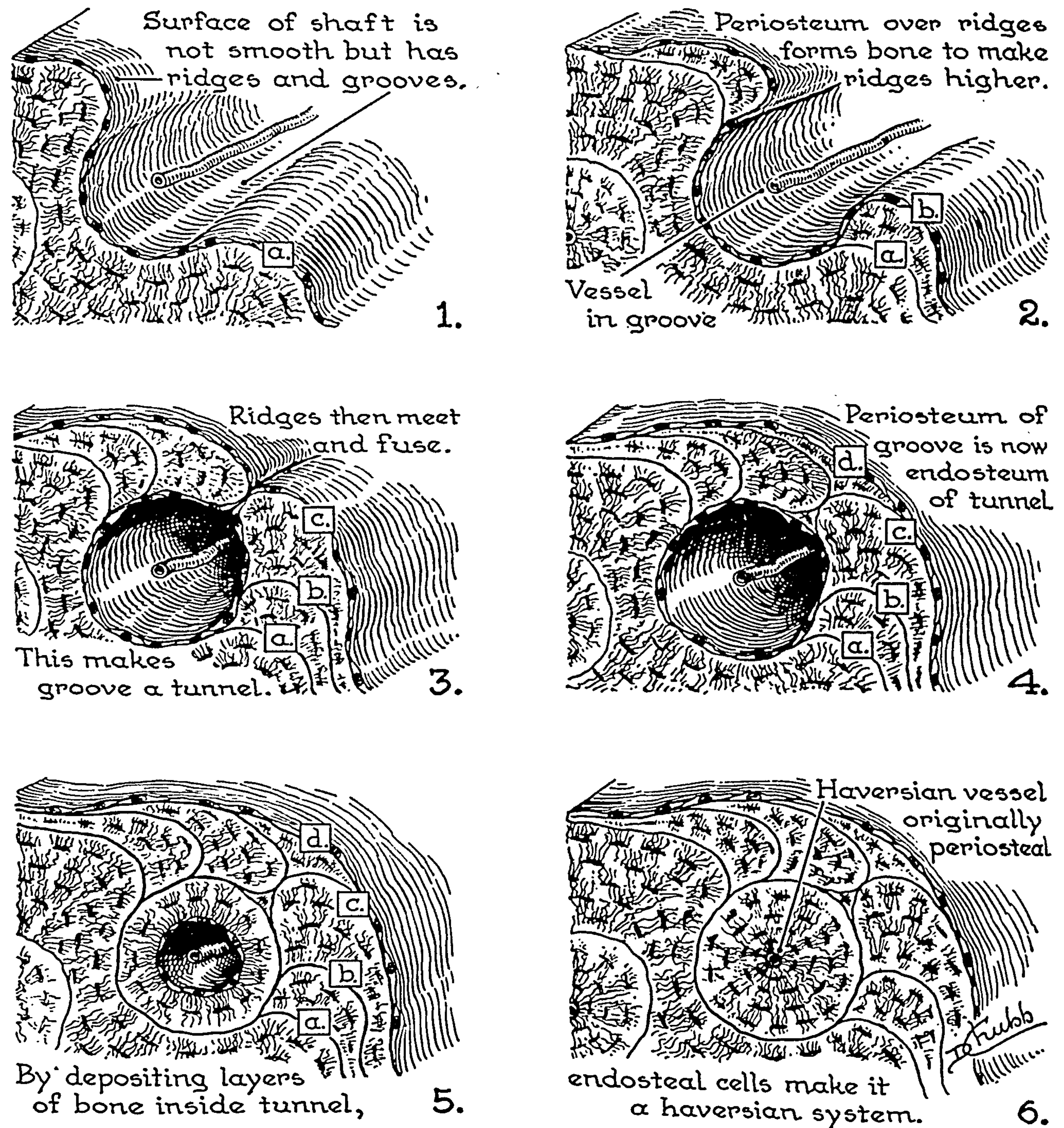


FIG. 65. How haversian systems are added to periphery of young shaft. From Histology, Arthur Worth Ham. Philadelphia: J. B. Lippincott Co., 1950.

ence or absence of periosteum with its cambium layer. In areas where new bone is forming there occurs an edematous infiltration followed by a multiplication of connective-tissue fibrils. Around the connective-tissue bundles a condensation of fibrils takes place; a true network is formed, and in this the preosseous substance is deposited. The whole is later calcified and the connective-tissue bundles are obscured by the deposit of salts.

Later investigators (23) have supported the mesenchymal theory and elaborated on the chemical mechanism of bone formation. Tissue which is to become the site of calcareous deposits contains an enzyme phos-

phatase which hydrolyzes the ester and sets free inorganic phosphorus. Activity of this enzyme phosphatase is dependent upon the hydrogen ion concentration. When the blood supply to bone is decreased the tissue undergoes calcification.

RESUMÉ

There are two or even three schools of thought regarding the agency responsible for bone formation. One favors the *osteoblastic theory*, which rests on the concise premise that the osteoblast is a specialized cell which has a monopoly on the production of bone and is an important factor in the survival or replacement of bone grafts and in the healing of fractures. These osteoblasts are most numerous in the innermost layer of the periosteum known as the cambium layer, which is adherent to the outer surface of bone, but they also reside in the endosteal layer of cells lining marrow cavities and extending into the haversian canals. The osteoblasts are therefore strategically located wherever their services are required.

A second group of investigators believe that the osteoblasts do not have bone-forming proclivities but that osteogenesis is the result of metaplastic change in connective tissue and may occur in any tissue. Presumably, certain connective-tissue cells, which are widely distributed throughout the body, retain their embryonal characteristics and these cells are responsible for new bone formation.

Possibly both theories are true. The osteogenic layer of the periosteum appears to be important for the normal appositional increase on the surface of bones during the growth period of mammals. New bone formation about a fracture or bone graft in a young mammal may occur from both the osteoblasts and certain of the connective-tissue cells which reside in, or migrate to, the area where their services are required. In older mammals the periosteum serves more as a covering membrane for bone and a source of nourishment for the outer cortex of the bone. Osteogenesis in fractures and about bone grafts in the older mammals may occur largely through activity of the undifferentiated connective-tissue cells in the area rather than from the osteoblasts in the periosteium, haversian canals and marrow cavities. In effect, nature may have more than one way or one agency to accomplish osteogenesis but the living elements or cells concerned in the process are probably quite similar whether we differentiate them as osteoblasts or undifferentiated connective-tissue cells.

An analogy for this specificity is often noted in the construction of other tissue materials in the body: melanin pigment is produced by melanoblasts, collagenous fibers, by the fibroblast, and the insulating myelin sheath about nerve axons possibly through activity of the Schwann cell etc. On the other hand, no one agrees concerning the specific cell responsible for the formation of elastic fibers, and some believe that it occurs as a sort of chemical precipitation with or without living cellular supervision.

According to Levander (24), some substance is liberated from a bone graft which acts as a specific stimulus to the mesenchymal tissue in the vicinity and initiates osteogenesis. Alcoholic extracts of bones (and fracture callus) stimulated the formation of bone and cartilage at the site of injection in twenty-two of his experimental animals. This is sometimes called the *specific stimulus theory*. Levander believed that only the periosteum of the growing skeleton has the power to form bone but after growth is complete new bone formation is initiated by mesenchymal cells.

It is noteworthy that most of the experimental observations recorded in this chapter were based on animal experiments rather than those on humans. In animals some tissues have regenerative powers or growth

properties after grafting and a tenacious ability to survive free transplantation, which are not seen in the more highly developed human tissues. One must emphasize, therefore, that the results of animal experimental work are suggestive but not conclusive. The final proof is the determination of how free grafts behave in human tissues.

C. M. Pomerat at the University of Texas and research workers in other Centers are beginning to study the behavior of adult human tissues in tissue culture. Undoubtedly this approach will add greatly to our knowledge of human tissues and their fate after they have been transplanted as free grafts.

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CHAPTER 16

Transplantation of Bone in Animals

The first successful autogenous bone graft in animals was recorded by Merrem (1) in 1809. He obtained healing of bone plates which had been removed by trepanation subperiosteally. There were probably many previous attempts at bone transplantation of which no written record was made. Von Walther in 1821 and Bernhard Heine in 1836 succeeded also in obtaining healing of trephined bone removed subperiosteally from animals in isolated instances (2). In successful animal homoplasty by Flourens (3) in 1843 a trephined slice of bone healed in; while in reimplanting pieces removed subperiosteally from the skull, rib, and tubular bones Klencke, Wiesmann, and B. Heine (3) met with failures through resorption and suppuration despite favorable conditions. Likewise, homoplastic and heteroplastic animal transplantations in the abdominal cavity ran an unfavorable course as reported by Middledorpf in 1852 and Jouck in 1853 (3).

Since these first attempts the literature on the subject has grown prodigiously and contains a wide diversity of confusing opinions regarding the best type of graft and the fate of the grafts after transplantation. At the end of the nineteenth century many investigators were of the opinion that under favorable conditions bone transplants and especially autotransplants remained alive for the most part. There were some authors, however, who held the opposite opinion (4).

In deference to the general abbreviated pattern of many publications today an author should digest the material which he presents in a condensed manner and not waste the reader's valuable time. It is difficult, if not impossible, however, to present the experimental work on animal bone grafting concisely in this manner and still retain some standard of accuracy in recording what the various experimenters actually did. Foreign articles are especially difficult to translate clearly into readable English because of different terms and modes of expression. I have used the terms and summaries as given by the various investigators in some instances instead of stating what I think they concluded. This is good from the standpoint of accuracy but does not always make for easy reading and clear understanding.

In this review on bone grafting in animals a sincere attempt has been made to indicate the type of graft used in the experiments, viz., whether autogenous, homogenous, heterogenous, fresh bone graft, frozen bone graft or graft treated with some preservative or exposed to heat before transplantation. An effort has been made also to state where the bone came from, whether it was cortical or cancellous, and the nature of the recipient

site. When one also adds the presence or absence of periosteum and some very weird maneuvers in transplantation the matter becomes still more intricate.

Many investigators in a single experiment buried autogenous, heterogenous, and homogenous bone in the fresh state, from the deep freeze, heat-treated and immersed in various preservatives. It is difficult to separate all of these experiments by a single man and include them in appropriate chapters and hence they are presented together in general as the work of a single individual.

This review represents samplings of the vast amount of experimental work on bone grafts in animals and is far from complete. It is presented as accurately as possible to provide a reasonably sound basis for future writers on the subject of bone grafting.

The reader with casual interest should confine himself to reading the summary on bone grafting in animals.

AUTOGENOUS AND HOMOGENOUS BONE GRAFTS

Early Experimentation

Julius Wolff (5), a student of v. Langenbeck, in 1862 presented the difficulties in transplantation of osseous tissue with special exactness. Since the external surface of a bone proves nothing about the life or death of the tissue, he sought an answer by vital staining of transplanted tissue from his test animal with madder dye. He obtained healing of autogenous bone fragments in replantation in the rabbit skull, and found red staining of such fragments in his test. Wolff believed that this red staining of the transplant showed it to have remained alive. He further concluded that maintaining the vitality of a whole transplant is possible but only in flat bone.

Ollier, a great French surgeon and physiologist, in 1867 is known as the first to have systematically examined bone grafts. He believed that a fresh autogenous or homogenous bone transplant, covered with periosteum, is kept alive when its own periosteum becomes quickly revascularized. His work formed the basis of much subsequent investigation (6). Radzimowsky (7) in 1881 indicated that bone transplants, with or without periosteum, evidenced only temporary viability. Bonome (8) in 1885 histologically examined bone implanted from the femur into the muscle of the back in a rat. He believed that the bone of even an autogenous graft dies but the transplanted periosteum retains its osteogenic properties.

Barth (9), an independent thinker, in 1893 and 1895 concluded from his histological studies that *all elements of both autografts and homografts of bone died whether the bone was transplanted with or without periosteum, and were replaced by host tissue.* Any bone graft becomes necrotic in all its parts and then is regenerated through osteoblasts from neighboring sound bone (creeping substitution). This belief that all bone grafts are absorbed and replaced by new bone matrix and new bone cells from the host bone is accepted today by most authorities.

While opinions differed, the general consensus from a clinical viewpoint was that living bone served better than dead bone. Axhausen (10) in 1908 held that living periosteum is essential to the viability of a transplant, while the bony tissue itself dies. The replacement of dead bone occurs chiefly from the periosteum of the bony graft.

In experimenting with rabbits (implants in skull bone) and dogs, Tomita (11) came to the conclusion that the greater part of the bone substance was lost through resorption, and new bone growth arose from the periosteum and marrow cells.

Axhausen (12) in 1909 transplanted homogenous bone with periosteum and endosteum into the soft tissue, using rats, cats and dogs, and noted that these transplants regenerated but not so noticeably as in

autogenous grafts. In the same year Albee (13) did a series of experiments, fusing the dog's spinal column with a free graft from its leg. The results were so promising that he was encouraged to start similar work on humans. He believed that transplanted autogenous living bone becomes a part of the osseous system but adequate blood supply and coaptation are required.

Several reports in the literature of 1912 are of interest. In working on young dogs, Macewen (14) observed that in resection of whole thickness of bone, leaving the periosteum, the gap is filled with new bone. He showed that the filling of the gap may come from two sources other than the periosteum, viz., from the epiphyseal cartilage pushing the cut fragment toward the gap, and from proliferation from the ends of the bone. Such experiments, in his opinion, have no bearing on the osteogenetic function of the periosteum. If bone is produced by a periosteal flap it is due to the superficial layer of bone having been raised with it. Macewen held that a cambium layer lies between the bone and the periosteum in young animals, and when the periosteum with the cambium layer attached is raised, it will produce bone.

McWilliams (15) observed perfect growing when autogenous osseous pieces, without periosteum, taken from the humerus were implanted in a defect in the ulna of the cat. An autogenous radial graft, without periosteum, healed in the humerus of the cat, and similarly a piece of the humerus transplanted into a radial defect was found to have healed in (72 days later). Autogenous and homogenous rib removed, without periosteum, and buried in the abdominal wall of the rabbit, healed by primary union, as did long autogenous strips of rib, without periosteum, also buried in the abdominal wall. McWilliams considered his experimental work suggestive and not at all conclusive.

Working with bone transplants in animals, presumably autogenous, Baschkierzew and Petro (16) in 1912 expressed the belief that bones without periosteum transplanted into soft parts are capable of regeneration. The chief source of regeneration of bone in soft tissue lies in the primary layer of granulation tissue surrounding the transplant. Murphy (17) maintained that contact with living bone is absolutely necessary for the life of grafts. All his transplants were employed while having more or less a covering of periosteum.

Brown and Brown (18) in 1913 were unable to reproduce bone from free periosteum transplanted into subcutaneous tissue and muscle, or from free bone transplants without periosteum, or from free bone with periosteum intact, transplanted into similar tissues. However, they were uniformly able to reproduce bone when it was transplanted in contact with living bone provided it was in a position where it had a function to perform; otherwise there was absorption. They believed that bone produces bone without the aid of periosteum. Periosteum may be helpful but is not essential.

Employing cats and rabbits Cotton and Loder (19) removed a portion of one condyle and replaced it with a corresponding part of the opposite femur of the same animal. Their specimens showed a practically uniform survival of the transplant when the technique was adequate—comprising real asepsis, fair approximation of the graft to its bed, and reasonable fixation of the graft. Essentially, bone corpuscles disappeared early in transplanted trabeculae and in the trabeculae of host bone for a short distance from the wound surface. Without any loss of substance in the bone from which the corpuscles have disappeared, this bone is rapidly and completely covered by a layer of new endosteal bone which unites with endosteal new bone of the host. The new bone is laid down by the activity of end osteoblasts in all portions of the grafts.

Some of the end osteoblasts represent the actively-proliferating covering membranes of the transplanted trabeculae. Practically no changes either of degeneration or proliferation in the transplanted articular cartilage occur, at least up to four weeks.

In 1914 two painstaking observers, McWilliams and Phemister, contributed to the increasing experimental studies on animals. McWilliams (20) reported that transplanted periosteum from a rib into soft tissue produced new bone. In 16 transplants of the fibula of dogs and one human, with periosteum intact, implanted in soft tissue and in contact with bone, 93 per cent were successful in his hands (21). Of 25 transplants without periosteum in animals, only 48 per cent showed good results. When small fragments without periosteum were grafted, 50 per cent of the transplants were successful, while a good result was obtained in 41 per cent of the transplants in which single larger pieces without periosteum were grafted. On the basis of his studies, McWilliams believed that the function of periosteum is to maintain the nutrition of the grafts quite apart from any osteogenetic function it may have. Contact with living bone was not necessary, the life of the graft depending on sufficient blood supply. He further concluded that bone of a size to assure viability should be transplanted with as much periosteum covering its surfaces as possible.

Phemister (22) removed about one-third of the ulna in dogs and replaced bone plus periosteum and endosteum, bone minus periosteum, bone minus periosteum and endosteum, boiled bone and periosteum alone in its bed. Osteogenesis in bone repair occurs from the inner layer of periosteum, from endosteum and to a much less extent from bone cells and fibrous content of the haversian canals. Viability of cells of a transplant is dependent largely upon their ability to get nutrition and to some extent upon their degree of cell specialization. The subsequent changes which the transplant undergoes depend upon its composition and location.

When bone is transplanted into a bony defect, the functional demand stimulates the surviving cells of the transplant to osteogenesis. Creeping substitution of dead cortex occurs by ingrowth of capillaries with dilatation of the haversian and Volkmann's canals, absorption of old bone, and deposition of new bone in its place.

Another conclusion by Phemister was that when periosteum and endosteum are left on, the transplant contains the greatest number of living osteogenetic cells. When periosteum is removed, osteogenesis and substitution occur from endosteum and a few surviving cells of the cortex; the process is slower. When both periosteum and endosteum are removed, union and substitution of dead bone are much delayed; then new bone formation from the few surviving cells is slight. Periosteum transplanted into ulnar defects and into subperiosteal ulnar resections failed to regenerate bone, but in subperiosteal resection with reimplantation of cortex either alive or after boiling, a layer of callus formed about the reimplanted portion. When bone is in contact with soft tissue, little proliferation or substitution occurs, and the transplant is gradually absorbed. Transplanted periosteum produces little or no new bone formation.

In a series of experiments, Mayer and Wehner (23) observed bone production after free autogenous periosteal transplantation from the tibial surface intramuscularly into the thigh of dogs, and into subperiosteal rib resections in rabbits. When a capsule was placed on the medial tibial surface near the upper epiphysis in dogs and in rabbits, there was no growth of bone after varying periods of days. In transplantation of bone without periosteum, with and without marrow and endosteum, with and without a

capsule, and with periosteum, the distant bone cells showed no activity, while periosteum showed active bone-forming ability. The same osteoplastic function was present for the endosteal cells of the medullary cavity as of the haversian canals. Macroscopically bone without periosteum can be effectively transplanted into muscle because specific bone-forming cells are transplanted along with it. Mayer and Wehner concluded that bone should be transplanted with periosteum in order to make possible inner union of the endosteum with the surrounding tissue. The majority of bone cells die, but a part can maintain themselves until vascularization of the transplant. The necrotic bone of the transplant is replaced by newly-formed young bone; the young bone cells resolve the necrotic bone and form new bone. Young newly-formed bone penetrates the old necrotic bone and substitutes for it. Young cells penetrate into old empty bone cavities.

In 1915 Phemister (24) reported on subperiosteal resection of the shaft of the tibia and humerus in dogs before closure of the epiphyseal lines. In all animals there was a good attempt at regeneration of the tibia and humerus. He believed that osteogenetic tissue must have been left because regeneration occurred.

In 1915 there also appeared a report on implants of periosteum in dogs and rabbits by Davis and Hunnicutt (25). They found that free periosteal transplants (fibula and tibia) did not produce bone in the majority of experiments even though osteoblasts were adherent to the transplants. If shavings of bone were left attached to the periosteal implants buried in soft tissue, new bone formation was definite. Removal of the periosteum had little, if any, effect on the nutrition of a bone. Furthermore, periosteum seemed to provide some protective influence against early absorption.

In the cultivation of small pieces of bone taken from mice, kittens, and rabbits, Dobrowolskaja (26) in 1916 observed that bone tissue is capable of producing a luxuriant growth *in vitro.* He further stated that the living elements of compact bone tissue are also capable of developing new cells. When bone is transplanted with its periosteum, according to his opinion, the growth is evidently more active. Bone should be connected with matrix bone to provide strength.

In Groves' experiments on adult cats, pegs of autogenous tibial bone and fresh and boiled homogenous femoral bone, driven into holes drilled in the tibia, became incorporated into the shaft of the bone in six weeks. Microscopically there was very little difference. The tissue of none of the pegs showed surviving cells; at the periphery there was encroachment by living bone. A homograft from the femur implanted in a gap in another cat's tibia showed the same result macroscopically and microscopically as when an autograft was employed. Homogenous tibial bone implanted in tibial bone showed firm bony union between the graft and the host bed, with considerable callus excess, accompanied by characteristic growth of new epiosteal, endosteal and haversian new bone in the graft (27).

Groves (27) summarized his experimental results as follows: The ideal graft is a piece of living bone used in its entire thickness. Any kind of bone graft gives better results when used whole than when broken up into small fragments. Fragments of living bone, unless closely in contact with vascular tissue, display no osteogenesis. Dust formed from living bones does not maintain its vitality. Cortical grafts are far better than intramedullary ones. An intramedullary graft, if small and loose, takes no part in repair, neither does it act as a splint. The success of a living graft very largely depends upon the extent of its contact with living bone, the accuracy of its apposition, and the

firmness of its fixation. Homogenous grafts, under favorable circumstances, act just as well as autogenous grafts.

Experimenting with autografts from the radius of dogs, with and without periosteum, Gallie and Robertson (28) in 1919 concluded that the presence or absence of the periosteum had no decided influence on the activity of subperiosteal osteoblasts. The periosteum is not osteogenetic surgically and should not be depended upon to assist in the production of new bone. The processes are more rapid when the graft is in contact with living bone than with muscle apparently owing to the greater supply of living cells. Absorption and replacement occur; the graft is covered with adherent newly-formed trabeculae; the osteoblasts lay down new bone. Homogenous bone transplants may act similarly to autografts.

In an experimental study of raw and boiled bone fragments (knee joint resection) buried in the thigh muscles of the same dog, Ely (29) (1919) noted that no boiled bone could be recovered after 150 days, while raw bone in one instance persisted for three years and seven days. Raw bone resists absorption better than boiled bone but it also is slowly absorbed. He concluded that both bone and marrow in buried fragments die. The marrow is then reformed by blood vessels, and a certain amount of new bone is laid down upon the old, especially along the margins of the trabeculae. The cartilage usually lives but slowly becomes eroded at its surface, and becomes thinner. In Ely's opinion, periosteum does not form bone. The portion of dead bone adjacent to it is more quickly vascularized and hence is the earliest seat of new bone formation.

Brooks and Hudson (30) in 1920 experimented with dogs, defects in the ulna being bridged by autotransplants, without periosteum and endosteum, and by homotransplants with periosteum and marrow surfaces intact. The homografts as well as the autografts may result in complete regeneration of the defect.

In experiments on rabbits, as reported by Klinkerfuss (31) in 1924, periosteal callus (produced in the ulna) was transplanted to costal cartilages after the perichondrium had been scraped off. Roentgenographically and by serial sections it was evident that callus grafts do not die but continue growing after transplantation. Callus grafts form new bone more rapidly and in greater amount than, and also persist as long as, solid bone grafts, and become quiescent at about the same time.

In the study by Ely (32) (1924) the knee joint in the cat was opened and a piece of lateral condyle removed and buried in thigh muscles of the same cat. According to stained sections the bone and its contained marrow died. About a week later vascularization of the bone marrow began and shortly thereafter new bone formation took place.

To determine the role played by the different bone tissues in bone regeneration, Rohde (33) carried out a series of experiments with periosteum, endosteum, and compact bone on dogs, cats, and rabbits. Radiologic, macroscopic and microscopic examinations were made. Rohde was able to substantiate powerful regenerative action of periosteum. As a result of his clinical experience and experimental work he concluded that bone-building power is found only in specific bone-building tissues (osteoblasts of periosteum and marrow endosteum). Metaplastic bone building from the usual connective tissue of musculature, the muscle septa, the tendons, the fascia and subcutaneous tissues does not occur. Bone formation in soft tissue is from the unusual remaining mesenchymal cells, which through traumatism, infection, toxic stimuli, or disturbances of metabolism, may abandon their indifferent stage at any time and commence to build bone.

In Wereschinski's experiments on young

rabbits, bone growth *in vitro* was negative, as was the cultivation of periosteum and endosteum. After autogenous and homogenous transplantation of small bone fragments, with and without periosteum, into the subcutaneous connective tissue and the intermuscular connective tissue of the abdomen, the new formation of osseous tissue was insignificant. In autoplastic and homoplastic transplantations of fibular sections into the bone marrow canal of the tibia, resorptive processes of the transplant occurred, and there was new formation of the bony trabeculae, which, though temporary, led to union of the fibula with the inner surface of the tibia. In fixation of live periosteum-covered autoplastic bone sections on the surface of the tubular bone, the results proved most satisfactory. The bone transplants fused with the bony layers after they had undergone a series of resorptive and reconstructive processes; a wholeness is formed which acts functionally and clinically with the best results (34).

Imbert (35) established that autogenous bone grafts behave differently depending upon the host tissue with which they are in contact. If placed in the same site of resection, bone grafts always produced repairment and bony proliferation. When implanted in soft parts, the grafts were progressively resorbed. Experimenting with the paws of dogs, he concluded in 1926 that autografts on bone other than the place of resection failed, while those *in situ* were almost constantly successful. This rule did not apply to young animals supplied with growing cartilage. Willich (36) (1926) concluded from his experiments with free autotransplants of bone in dogs that the endosteum and the bone marrow are just as important in bone formation as the periosteum.

In a new method of continuous microscopic study of bone growth in the mammal, Sandison (37) in 1928 observed new growth from an autogenous transplant of bone marrow, with endosteum, placed in a transparent chamber in the rabbit's ear. The tissue which precedes the first formation of bone has an appearance very similar to that of connective tissue, and it develops in regions in which the latter is present. The fibers of the early bone tissue are less sharp in outline, less transparent and are arranged in heavy bundles running in many directions. These fibers are connected with the highly granular region of new bone with clear interspaces between them. Small highly-refractile granules extend into the bundles; these becoming closely packed encroach upon clear regions replacing some of them, and narrowing others. There is a highly-refractile edge at the periphery of each oval or circular space. The granules finally coalesce, forming a hyalin-appearing homogeneous ground substance. Mature bone cells contain an irregularly-shaped dark circle instead of sharply-outlined nuclei. In some regions where bone is extending outward to form new bone, there is a process of resorption. Howship's lacunae occur throughout the tissue.

deJong and van der Kemp (4) transplanted into the dorsal muscles in rabbits autoplastic parts of bone—with periosteum and marrow, without periosteum and with marrow, without periosteum and without marrow, and with periosteum and without marrow. They also replanted osseous parts into the tubular bone after peripheral resection and after continuity resection. In the initial phase, when strong proliferation of the osteogenic tissue occurs, osteoid tissue of an immature appearance is formed through calcification of interstitial substance and a perceptible destruction of cells. In the following period, when the period of strongest proliferation is past, the osteogenic tissue forms osseous tissue, which is very regular and is distinguished only by somewhat larger cells—with oval nuclei and strongly stained interstitial substance—from normal osseous

tissue. Finally, the activity of the osteogenic tissue extends back to the borders of normal growth.

In 1928, Dorrance (38) passed osteoperiosteal bone grafts from the tibia into subcutaneous tunnels in the dog's jaw. Each end of the graft was placed over roughened bone and held in place by suturing soft tissues. None showed complete union on both sides. In repeated experiments (1929), when the grafts were sutured to denuded bone, three completely successful grafts resulted.

Kornew (39) in 1929, working with rabbits and dogs, transplanted autogenous bone grafts from the ulna, radius, fibula and tibia as follows: between the spinous processes of vertebra, into defects of the long tubular bones of the ulna and radius, and into the fibula. Some of the grafts had periosteum attached and some were denuded of periosteium. The grafts in the tubular bones and in the spinous processes all proceeded to heal quickly. The osteocytes in the transplants degenerated and the grafts were replaced by new bone formation which resulted in restoration of the transplant. Kornew, surprisingly, reported that in young animals longitudinal growth of transplants as well as growth of basic ground bone *occur interstitially*.

Pollock and his colleagues (40) in 1929 transplanted fresh and boiled devitalized segments of presumably autogenous ribs, with the periosteum intact, into the muscles of the back, the knee joint, and the abdominal cavity in dogs. Additionally, fractured living transplants were enclosed in an onion or collodion membrane to prevent ingrowth of cells and blood vessels from the tissues of the animal and to permit dialysis of nourishing substances. The animals were killed at intervals of 8 to 42 days for microscopic examination. Pollock *et al.* concluded that new bone formation in rib transplants occurs only in segments transplanted alive and only when the transplant becomes surrounded by well-vascularized fibrous tissue. Death of the transplant invariably takes place when a membrane prevents ingrowth of fibrous tissue and blood vessels. When new bone formation occurs, the cells in the lacunae of the transplant, at least about the peripheral areas, contain staining nuclei, and osteoblasts are present in fairly large numbers. Absorption of bone begins as new bone is formed to replace it.

Kartaschew (41) in 1930 transplanted fine autogenous bone segments into adult rabbits in contact with bone and in soft tissue sites. Some grafts had periosteum and bone marrow and some were transplanted without periosteum and marrow. The healing of free autogenous bone grafts depends upon the presence of periosteum and endosteum. The transplanted bone is completely destroyed, with subsequent re-formation by new bone. The main source of this new bone formation is the periosteum, endosteum, and marrow. A good blood supply in the host tissues favors reconstruction of the transplant, and especially favorable conditions of nutrition for the endosteum and marrow lie in the transplantation of fine pieces of bone rather than large segments. A metaplastic bone formation also occurs from the surrounding young connective tissue.

In Burman and Umansky's experimental study (42) (1930) free periosteal grafts from the tibia of young rabbits were stripped of periosteum, and the tendon of the tibialis anticus was lifted out of its bed and scarified. The synovial sheath was not permitted to remain. The graft was sutured to the tendon near its insertion and put under continuous functional stimulation. At varying intervals after operation the specimens were examined grossly and microscopically. Where the cambium layer was absent, no bone formation was seen except in one instance. The new-bone formation was first observed about two weeks after operation and was a typical periosteally-formed fibrous bone. In a few in-

stances endochondral new-bone formation was evident. Bony nodules, spirals or large osseous outgrowths were often felt along the course of the tendon, which was surrounded by a fibrous investment. The tendon showed a fibrous change. The coarse bony trabeculae of new bone infiltrated the fibrous tissue.

An autoplastic cortical bone graft transplanted aseptically into cortical bone in young dogs, with perfect mechanical adaptation and fixation, as studied by Davison and Kraft (43) in 1931, showed healing by primary intention, with preservation of the vitality of the graft.

Later Experimental Work

Haldeman (44), of the University of California Medical School, in 1933 compared, radiographically and microscopically, different types of grafts—fibula, periosteum alone, osteoperiosteal grafts, and cortical grafts with or without periosteum—in rabbits. The periosteum appeared to him as the most important part of the bone graft in respect to union of fractured bone and survival of the graft.

Imbert (45) (1933) reported a study of numerous sections of bone grafts removed and examined at different stages of their evolution. An autogenous bone graft buried in the site of resection loses the totality of its osseous cells; all the corpuscles are empty. At the end of some months, if a fragment of the graft is broken off, each corpuscle is found to include a living cell. The osseous fragment has been completely repaired. New cells appear, the first of which have consumed the calcareous substance and opened up large paths of communication. The next appearing cells have occupied these empty spaces and built a new formation of osseous tissue around them. The alternating evolution of life of the tissues has occurred in two different forms. There has been replacement of dead osseous tissue by living tissue. In the first place there is destruction, in the second phase, reconstruction. The two phenomena belong to the same process but are not produced in the entire thickness of the graft at the same time. Certain haversian systems have been consumed, then reconstructed, while others stand still.

In free autogenous bone-marrow and endosteal grafts placed in the resected portions of the fibula in dogs, McGaw and Harbin (46) (1934) noted radiographic indications of the formation of a new medullary canal and cortical condensation. The new bone was well fused to the shaft of the fibula. Microscopically, the defect was filled in by newly-formed bone which had apparently developed *in situ* rather than by proliferation from the ends of the fibula. McGaw believed this to be the first specific evidence relating to the activity of bone marrow and endosteum.

In a series of experiments by Keith (47) (1934) shavings of living bone and from the shaft from which the periosteum had been removed and the marrow and endosteum curretted out, were replaced in contact with bone in young and old dogs. Bone fragments, removed subperiosteally and retaining marrow and periosteum, were boiled or frozen in liquid air for ten minutes before being cut into shavings and then were replaced in contact with bone. Shavings of bone removed by subperiosteal dissection and boiled for ten minutes were replaced in a periosteal tube. Periosteum left after subperiosteal dissection of bone was closed. Shavings of cortical bone were replaced in rectal muscle. From subsequent observations, Keith concluded that a large number of osteogenic cells survive, particularly around the periphery of a mass of shavings, and this results in the formation of a considerable amount of new bone. In adult animals new bone formation was slow and scanty. The tendency to proliferate and form new bone in these small grafts appears to be dependent upon the presence of living osteogenic cells in the

grafts. Intact blood supply of the bed of the graft and the cambium layer of periosteum are important for regeneration. There was no evidence that metaplasia of other connective-tissue cells to bone-forming cells plays any part in new-bone formation associated with bone grafting.

Stewart (48) in 1934, in experimenting with dogs, filled radial defects with a mixture of lime salts consisting of tricalcic phosphate and calcium carbonate, in a bag made of fascia, a mixture of equal amounts of salts and bits of muscle, and a gelatin capsule containing salts. A carpal defect and a defect in the astragalus were also filled with salts. All specimens were examined radiographically and sections were obtained for microscopic study. There was failure of regeneration of the shaft in all instances.

In experiments on dogs, Bisgard (49) (1934) noted that autogenous costal bone, placed in the perichondrial sheath, gave support by fusing together to form a solid bone structure. This suggested its use in humans where much cartilage must be removed.

In Shands' experiments on dogs, a hole bored from the outer surface of the upper portion of the tibia into the medullary cavity was filled with calcium salts. All of the defects examined in from three to eleven weeks after operation, contained solid bone. More bone was found in the control defects than in those filled with calcium salts. In defects in the ulna calcium salts appeared to stimulate bone formation; in the spine they had no such action and appeared rather to exert an inhibiting influence (50).

A cylindrical piece of bone was removed in Bahls' experiments and reimplanted in the tubular bone of dogs; examination being made after fourteen days, five to six weeks, eight, twelve, and eighteen to twenty-one weeks. As perceived in the behavior of the periosteum and endosteum, the results of transplantation are essentially influenced by the living process in the bone transplanted along with it (51).

In another series of experiments with rabbits, Bahls (52) removed marrow from the cortical defect in the femur diaphysis and filled it with autogenous spongiosa from the ilium. The spongiosa thus transplanted died off and was replaced by new bone. The regenerated transplants in the bone marrow space were resorbed. Bahls believes that despite the dying-off process these transplants exercised an unusually strong stimulation on the new-bone formation.

Levander (53) of Sweden in 1938 transplanted hard bone tissue stripped of periosteum into soft tissue in rabbits. The tissue reactions showed that new bone tissue is formed from the mesenchymal tissue in the areas surrounding the graft. He also injected alcoholic extracts of autogenous bone and callus intramuscularly, and noted the formation of cartilage or bone at the site of injection. His opinion was that all grafted tissue died and newly-formed bone close to the graft was produced as a result of the permeation of the surrounding host vascular connective tissue by some specific osteogenic substance originating in the graft and activating the non-specific mesenchymal tissue. Injection of extracted callus sometimes produced osteogenesis. Similar experiments repeated in 1940, employing bone marrow freed of bone spicules as far as possible, gave similar results.

Tosatti (54) (1940) placed an autogenous piece of renal parenchyma between the ends of a defect in the ulna produced in dogs by subperiosteal resection. Seven months later the animals had perfect use of their legs. The renal pelvis was capable of healing the bone defect. Radiographic and microscopic examinations were carried out. Newly-formed bone solidly united the two ends.

In a series of experiments by Pollock and Henderson (55) (1940) bone grafts, with and without periosteum, applied in the femora of

the same dog were examined radiographically and microscopically in 99 to 113 days. When periosteum had been removed, a fibrous periosteum developed from surrounding connective tissue. The inner layer of fibrous periosteum showed evidence of the formation of young bone. When the periosteum had been retained in the bone graft, it was found to be inactive or absent. Pollock and Henderson concluded that no advantage is gained by retention of the periosteum in bone grafting.

Horwitz (56) in 1942 reported on grafts implanted from one tibia to the other, from one ulna to a defect in the other ulna, and bone transplants from the iliac crest to tibial sites in the same rabbit, and the wounds packed with sulfanilamide crystals. Observations at intervals of two, four, six, and eight weeks (and a limited clinical experience with five patients) indicated that local implantation of sulfanilamide crystals has no apparent adverse effect on the fate of transplanted bone.

In studies made by Schram and Fosdick (57) (1943) fibrous union resulted when bone and periosteum were removed from the radius in dogs. Similarly, when synthetic bone paste (consisting of chemical salts in proportion to their content in bone and a gelatin base) was placed in a radial breach, there was partial bony regeneration. When the movement of the synthetic paste and of the surrounding soft tissue was restricted by use of tantalum foil, complete bony union was obtained.

Hoyer (58) (1946) noted that when autogenous ground bone paste was inserted into the periosteal sheaths from which segments of the fibula had been removed in rabbits, bone production was much more rapid in three weeks than from bone chips; vascularization being facilitated.

Grafts of living embryo chick bone—os frontale—were transplanted by Hancox (59) in 1947 to the chick chorioallantois. These grafts survived and were rapidly vascularized, a complete circulation becoming established *within the live fragments.* Boiled fragments did not have the same appearance. The matrix was shrunken and distorted with remains of cells and connective tissue; the reaction of the host tissue was rather slight. Hancox believes that osteogenesis occurred in living bone. It seemed likely to him that short capillary buds developing from the host join up with vessels preexisting in the transplanted fragment.

In a series of experiments Abbott and his colleagues (60) implanted autogenous cortical and cancellous bone in cortical defects in the shaft of the tibia, and grafted the cancellous portion of the ilium, rib, and cortex of the tibia in a defect of the shaft of the radius in rabbits. Autogenous cortical or cancellous bone was placed on the anterior and lateral surfaces of the patella, femur, and tibia, and in iliac osseous defects in dogs. Cortical bone and bone from the spinous process or rib were placed in split spinous processes, and cancellous iliac bone placed at the site of osteotomy of the tibia also in dogs. Abbott concluded that in any graft, whether it be cancellous or cortical, the only elements which survive and which have osteogenetic power to any degree, are the cells that form the so-called endosteal layer; and to a lesser extent the cambium layer of periosteum.

In McKelvie and Mann's (1948) study on autogenous chipped cancellous iliac bone transplanted into the tibia of the rabbit, sections were made at intervals of one to 120 days after operation. The osteoblast is the intermediary between cellular and semisolid and solid phases of the formation of new bone. McKelvie and Mann were able to identify osteoblasts persisting in young osteoid in a form other than that of the osteocyte. They called these cells "matrix cells," because they believe that they are concerned with the uncalcifiable part of bone tissue. At

the fully mature osteoblast stage the cell may persist as an osteocyte, or become a matrix cell. As soon as the semisolid and solid stage is reached, osteoid tissue forms around the cells. They advance the hypothesis that the enzyme, alkaline phosphatase, is concerned more with the formation of the fibrocollagenous framework or matrix of bone itself than with the impregnation of this framework with calcified salts (61).

In experimental work by Pfeiffer (62) (1949) an acid alcoholic extract of bone did not produce development of bone when it was injected into the testes of 33 mice. As fragments of bone marrow produce bone, Pfeiffer concluded that the marrow reticulum cells survive grafting and produce bone.

In experiments on rabbits carried out by Heinen, Darbs and Mason (63), intramuscular injections of irritating substances such as alcohol alone, calcium chloride, or mixtures which contain these irritants, were frequently followed by ectopic chondrification and ossification. Intramuscular injection of extracts of bone, cartilage, or muscle does not produce cartilage or bone more frequently or more extensively than does the extracting fluid alone. Heinen and his associates concluded that the existence of a specific osteogenetic substance ("osteogenin") in extracts of skeletal tissue cannot be accepted as proved.

Hutchison (64) in 1949 did a series of experiments in which bone transplants were introduced into the anterior chamber of the rabbit's eye. As long ago as 1877 Connheim (65) appears to have originated the method, which was described by Schochet (66) in 1920. The anterior chamber of the eye is an ideal site for buried transplants because the transplant is visible and it is not in direct contact with blood vessels; moreover, the transplant grows under optimal conditions of body temperature and nutrition. Perhaps it is too ideal—since it is better than other sites in the body from the blood vessel angle and visibility standpoint, but it cannot be placed in contact with bone as in usual clinical use. Living cells found in such a transplant, however, cannot come from adjacent bone.

Hutchison used 50 transplants from the ilium, autotransplants in one eye and homotransplants in the other eye, all fresh. The rabbits were unselected mixed stock. The animals were killed at intervals of 10 to 180 days and the transplants were radiographed, sectioned and examined under the microscope. The autotransplants up to 180 days resembled normal rabbit bone with living cells. There was a well-marked periosteum present, which appeared to be formed by the host tissue cells. The homografts at 180 days and before were dead, and the grafts formed sequestra, with no bone cells or periosteal formation. The cells of the cartilage which was present in the rabbit ilium, however, appeared to be living in both the auto- and homografts.

Thus, the bone autografts survive but bone homografts become sequestra. Cartilage cells live in both cases. The peripheral cortical layer of the autografts is probably formed with the periosteum from the host cells. In these experiments the autotransplants survived irrespective of the presence of periosteum. In autotransplants some of the cells in bone grafts survive but peripheral cells probably come from the host. As stated by Hutchison, there is no authentic record of the histological survival of the bone homograft. He believes that a host reaction develops before vascularization of the graft can occur. Still today opinions regarding the source of bone regeneration differ. Some hold that periosteum and the grafted bone are the sole cause of regeneration; others believe metaplasia in the host connective tissue to be the chief cause.

Up to the present time it is a matter of conjecture as to what exactly happens in transplanted autogenous bone grafts. As

Hutchison aptly states the problem, does the bone graft continue to live, or does it become a dead framework? If new cells grow into a transplant, are these osteoblasts from existing bone or metaplastic cells from surrounding connective tissue? What is the comparative value of cortical and cancellous bone? Is periosteum a necessary factor? Does one large graft succeed as well as many small pieces? And what is the comparative success of auto- and homografts? In order to study these problems many workers have embarked on extensive series of animal experiments in which bone tissue was transplanted to various sites and its fate studied.

Reynolds and Oliver (67), of Washington University School of Medicine, in 1950 experimented on dogs, removing a block of autogenous bone from the tibia to implant in the leg. There was no evidence that any of the bone elements of an autogenous transplant live or retain osteogenetic power. Fixation and replacement of autogenous and homogenous bone grafts were accomplished by appositional growth of host bone. Reynolds and Oliver believe that autogenous grafts are superior to homogenous grafts, because the early phase of healing is more rapid and uniform, due to less host reaction and tissue specificity. They could find no microscopic difference between the autogenous and homogenous bone at the end of ten weeks following transplantation.

In the ulna defect in old and young rabbits was placed a split diaphyseal cylinder, with or without periosteum, from the same ulna in a series of experiments by Vainio (68) in 1950. The bone graft was placed in fragments of host bone, both with convex surface outward and with it in toward the marrow. In another series one half of a bone graft from the ulna was stripped of periosteum and in the other half periosteum was left intact, and then the bone was replaced in its former site. Radiographic and histologic examination at different stages of regeneration were followed. Vainio concluded that the periosteum of an autogenous compact bone graft is of essential importance in bone regeneration. This he considered to be due to its ability to survive and to proliferate after transplantation together with bone, and also due to the fact that the periosteum of the transplant may have an organotropic effect and more qualifications than the other elements of bone for the organizing activity which determines the shape of the regenerated bone.

Lacroix (69) in 1951 planted bone autografts under the renal capsule and concluded that all the transplanted bone had been absorbed and plaques of new bone were formed from the host tissue. There were also survival, proliferation and osteoblastic formation from the periosteum, haversian canals, and endosteum.

Odell, Mueller and Key (70) in 1951 compared the differences in the uptake of radioactive phosphorus (P^{32}) by autogenous fresh, boiled and frozen grafts from the dog's tibia from the superior medial aspect of which periosteum had been stripped. Merthiolate bone from a bone bank was similarly tested. They observed that autogenous bone becomes more radioactive than the other varieties. The uptake of radioactive phosphorus by inlay autogenous bone grafts is more rapid than the uptake by similar grafts of boiled or frozen bone. The uptake of radioactive phosphorus by autogenous bone chips intramuscularly buried is more rapid than that by frozen or boiled bone similarly buried. Merthiolate bone takes up less radioactivity than boiled or frozen bone.

Lindahl and Orell (71) (1951) injected white mice subcutaneously with extract of bone, and noted no formation of bone or cartilage after six weeks. When alcoholic mixture of bone pulp from the extremity of a guinea pig was injected intramuscularly into guinea pigs, microscopically there were no signs of bone or cartilage formation in 10

and 12 weeks. They came to the conclusion that the occurrence of a bone-producing factor, not bound to the life of the bone cells, must be held as certain, but that in all probability this factor is not to be found in the extracts about which reports have been made in the literature.

In experiments with 8 dogs by Ford, Lottes and Key (72), an autogenous free rib graft was flexed and its ends sprung into drill holes in the ilium, and its position under strong pressure was maintained by its own elasticity. The dogs were killed at weekly intervals to 6 weeks, and then at 8 and 19 weeks. If juxtaposition and relative immobilization are maintained, bones tend to unite whether or not their surfaces are pressed together. In their experiments pressure made little difference in the manner or rate of union. New bone which appeared early arose from cancellous bone inside the host, to fuse the autogenous bone grafts to iliac bone. Necrosis, indicated by empty lacunae, occurred in autografts and in host bone adjacent to defects made for insertion of the grafts. Absorption of bone grafts and ingrowth of fibrous tissue, new blood vessels and new bone occurred simultaneously, beginning at the base of the bone grafts, inside the host, and proceeding upward. The presence or absence of pressure seemed to make little difference in these processes.

Minor variations in the amount and consistency of callus which appeared were felt to be due to a difference in the gaps which had to be bridged on the two sides and to better fixation of grafts on the pressure side. There was no evidence that the bone grafts retained any viability or that any new bone arose from them.

Ray and his colleagues (73) worked with various bone-grafting materials implanted in the anterior chamber of the eye in guinea pigs and rats. Basic calcium phosphate apatite crystals, resembling the inorganic crystals from natural bone, injected into the anterior chamber of the eye produced no bone formation. The studies furthermore confirmed the fact that homogenous grafts of embryonic bone and autogenous grafts of fracture callus and adult cancellous and cortical bone can survive and proliferate in a suitable ectopic site. Frozen or lyophilized embryonic bone will survive and grow when implanted in an ectopic location.

Implanting a compound of ground bone (compact and cancellous) mixed with whole blood and powdered gelfoam on the chorio-allantoic membrane of fertilized hen's egg, Swanker and Winfield (74) noted no revascularization of compact bone fragments but observed progressive ingrowths of capillaries in the cancellous fragments. These findings confirmed those of Abbott and his associates.

An entire section of fibular bone in rabbits was removed and reimplanted with gelatinized bone into bony defects by Swanker Winfield. In the control leg the fragment was replaced while in the other leg the fragment embedded in autogenous gelatinized bone was inserted into the defect. In all instances healing progressed more rapidly when bone embedded in the gelatinized bone mass was utilized. A 60 per cent reduction in healing time was obtained.

In further study Hutchison (75) in 1952 reported on the behavior of autografts and homografts of bone when transplanted into muscle, bone, and the anterior chamber of the eye in rabbits. He ascertained that transplantation does not release the growth potential of the grafted tissue, even in young grafts, and "either the previously determined inhibition is still inherent in the graft or is imposed on the graft by the tissue hierarchy of its new milieu." His conclusions were as follows: Autografts of bone, after the initial period of devitalization, recover and survive as active living bone. Homografts of bone die and if transplanted into muscle the sequestrum may finally be absorbed. If implanted in bone they are replaced by new

host bone. The mature osteocytes of autografts degenerate in the early stages and are replaced by osteoblasts formed by metaplasia of cells of soft tissue elements of donor bone. Both osseous autografts and homografts induce osteogenesis in the connective tissues of the host. Homografts implanted in muscle or in the anterior chamber of the eye die.

Because of the very contradictory opinions regarding the ability of homografts and heterografts to replace the autografts Axhausen (76) (1953) carried out a series of experiments on dogs to obtain further factual evidence. Osseous auto-, homo- and heterografts, both fresh and preserved by cooling, were implanted into soft tissues as well as in bony sites. Histological examinations were made in varying periods of time ranging from the eighth to the seventy-fifth day. He pointed out that the biological value of different bony materials for surgical implanting depends in essence on the actual manner of taking the graft, on the osteogenic power of the graft, and the speed of bony repair. He concluded: "The fresh autograft is from the biological point of view unquestionably the best by far." The less favorable effect of homografts and even heterografts is due to the characteristic specificity of tissue, the individual, and the species. There is some compensation in the technical advantages of the bone bank. When the purpose of the transplant is to fill up bony cavities, all sterile bony substance produces good results in a bony bed. In the repair of the continuity of the long bones, autogenous bone grafts still offer the best chances of success.

PRESERVED BONE TRANSPLANTS

Ollier (77) (1867) is cited as referring to the value of refrigeration in the preservation of bone when he determined that rabbit bone kept at −1°C. (30.2°F.) gave a growth of bone four times greater than that of rabbit bone kept at 5 to 10°C. (41 to 50.0°F.). He also commented on the practicality of using cadaver bone in human bone grafting.

In his comprehensive experiments, Groves (27) (1917) noted that when homogenous femoral pegs were boiled for a half hour and driven into the femur of a cat, these pegs as well as fresh autogenous bone pegs all became firmly incorporated in the shaft of bone. No difference could be detected between any of them. There were no surviving cells in any tissue of the peg. Groves concluded: "Dead bone grafts are certainly inferior to living when used to fill defects in the long bones, but under favorable circumstances they become strongly incorporated in the living skeleton."

Nageotte (78) in 1918 demonstrated ossification in the region of some transplants fixed in alcohol. As we have seen, Ely (1919) noted that autogenous boiled bone fragments buried in thigh muscles of the same dog disappeared earlier than raw bone, not resisting absorption as well as the latter type.

Gallie and Robertson (28) (1919) included experiments on bridging gaps in dogs with strips of autogenous boiled (for ten minutes) bone, examination being made at intervals of a week. Where good fixation of parts took place, dead bone was united to fragments, and circulation was reestablished. The cells of the living bone responded to the stimulus provided by the dead bone. There was successful bridging of the gap, the dead bone being absorbed and replaced and the area ultimately filled up to normal thickness of the shaft of the bone. By the time dead bone was completely absorbed, its place was taken by a mesh of new cancellous bone.

In the resection of a piece of adult rabbit rib, Christophe (79), of the University of Liege, in 1923 implanted a homogenous rib fragment from an adult rabbit of a different breed. The costal fragment was fixed by boiling and then preserved in alcohol at 95°C. The devitalized graft became attached

to the stumps of living rib bone. In examination at various intervals after implantation, the graft was eventually found to be intact, without the macroscopic appearance of resorption. Callus formed in union. Microscopic sections, as noted by Christophe, showed the osteoblasts in rehabilitation. The results were also confirmed in experiments on dogs.

Attention was called by Haas (80) (1923, 1925) to the fact that after death the various cells are able to subsist for a limited period on such nutritive substances stored within them or surrounding them. Practical application of this principle was tried in experiments on dogs. A fractured metacarpal bone was kept at room temperature, and another in physiologic salt solution at 39°C. The fragments were united and buried in the muscles on opposite sides of the back in the same animal. In 5 weeks microscopic examination showed the cells in the bone to be alive. The regenerative processes can be retarded but not completely repressed by exposing the bone to a freezing temperature. Cells are able to form callus, even after being kept in physiologic solution for 19 hours. Haas suggested that the preservation of bone in cold storage would be more efficient in prolonging the survival period of cells.

Rohde's experiments (1924) on rabbits, cats, and dogs concerned presumably autogenous implants of cold-preserved bone (dead), bone deprived of living cells by autolysis, and bone with the caseous and chalky masses of calcified tuberculous lymph glands inclined to ossification, placed in the soft parts of the same animal. The experimental work with boiled bone pieces seemed to confirm the absence of metaplastic bone formation in the connective-tissue layer as traceable to the fact that fixation of organic and inorganic constituents of bone (with a small fibrous layer) by boiling makes difficult the progress and substitution for the granulation tissue of the bed. Living osteoblasts are absent. Experiments with autolysized bone and bone ash explained the properties of the contained bone constituents and their effect on the tissue bed (81).

In further experiments by Rohde (82) on rabbits, autogenous bone implants preserved for 14 days after removal were buried in a traumatized or infected bed of muscle, fascia, tendon and subcutaneous tissue of various regions. There was no metaplastic bone formation on the part of the connective tissue of these types of grafts in observations to 5 months. In Rohde's opinion, soft tissue ossification in a site other than the original one is related to unused left-over mesenchymal cells, which can always develop from their stage of indifference through traumatic, infectious or toxic stimuli or through disturbances in metabolism, into bone-forming cells and bone. The resorptive processes of dead bone implanted in the soft tissues or osseous elements are very slight. He suggested that specific osteoplastic tissue behaves differently relative to resorptive processes in bone than the usual connective tissue.

Imbert (83) (1925) observed that none of the autogenous implants in dogs with calcined grafts, with decalcified, alcohol-preserved and boiled grafts, gave successful results. A graft is influenced by the conditions of the milieu in which it is transplanted. Imbert concluded that freshly removed grafts constitute a material of bone reparation superior to that provided by grafts killed either by heat or by chemical means.

In Pollock's experiments (1929) on segments of ribs, with periosteum intact, implanted in muscle of the back, the knee joint and the abdominal cavity in dogs, boiled and unboiled transplants appeared exactly similar after having been enclosed in collodion membrane and buried for 8 to 42 days. In neither type of graft did healing occur (40).

When autogenous boiled bone was used for shavings, Keith (84) (1934) observed that

there was no invasion of the mass by osteogenic cells from the ends of the fragments, and no evidence of metaplasia of surrounding connective-tissue cells to bone-forming cells. Thus, he concluded, boiling for 10 minutes kills the cells and prevents osteogenesis.

Stewart (85) (1934) replaced segments of one radius, boiled for 10 minutes, in the radial defect in the same adult dog. There was no production of new bone within or about them. When small fragmented live bone grafts were transplanted, new bone growth occurred. Lime salts placed in a bone defect with either traumatized muscle or fascia did not serve as a source of available calcium resulting in super-saturation of connective tissue and regeneration of missing bone.

In experiments on rabbits and dogs, Inclan (86) of Havana showed that bone kept in citrated blood and refrigerated could be transplanted from one animal to another without local or general reactions. When primary healing occurred, these transplants would act like fresh autogenous bone. Histologically, the bone cells and bone tissues vary very little or not at all from preparations of fresh bone.

Working with rabbits, DeBruyn (87) in 1947 placed homoplastic and autoplastic transplants of bone, bone marrow, and periosteum, fresh and devitalized by freezing in liquid nitrogen or dry ice, in the thigh musculature. He found that freezing seemed to destroy the bone cells and bone-forming substances.

In an experimental study on the use of bank bone, Kimball (88) in 1949 transferred homogenous and heterogenous fresh, frozen and boiled bone to the anterior chamber of guinea pigs' eyes. Survival of transplanted homogenous bone and the initiation of osteogenesis by heterogenous bone were demonstrated. Kimball also showed that fresh bone and bone frozen up to 9 days had about the same ability to initiate osteogenesis whether homogenous or heterogenous. In his opinion freezing simply prevented the death and disintegration of the cells for variable periods so that these cells were able to retain their bone-forming substance until transferred.

Kiehn, Friedell and MacIntyre (89) in 1948 employed the metabolism of radioactive isotopes (P^{32}) as a measure of viability of immediate autogenous bone, fresh and frozen, and cartilage grafts. These experiments indicated that bone existed as a viable tissue from the time of transplantation, forming its own blood supply and integrating itself as a vital part of the system. Refrigeration may not depress the recovery of transplanted bone beyond a short initial stage.

Autogenous fresh and boiled iliac bone chips (both medullary and cortical) stripped of periosteum and muscle, were frozen and implanted in the subcutaneous tissue of the anterior chest wall of adult dogs. Autogenous frozen iliac bone grafts, Kiehn and his colleagues (90) concluded, do exist as viable tissue when implanted after storage for as long as 6 weeks, for they exhibit metabolism and incorporation of radioactive phosphorus (P^{32}). On the basis of their experiments they believe the assumption to be justified that there is no tissue-fluid exchange to and from the graft which does not become integrated as part of the body. Boiled iliac grafts buried in subcutaneous tissue differed from the viable grafts, both frozen, in that they did not become intimately associated with the surrounding tissues of the animal.

Reynolds and Oliver (67) implanted autogenous bone grafts from the tibia, homografts from a merthiolate bone bank, homogenous frozen bone (−20°C.), inlay grafts of cortical bank bone, and homogenous boiled bone in defects in the legs of dogs. Examinations were made at weekly intervals for 10 weeks. The specimens were decalcified. Fixation and replacement of both

autogenous and homogenous bone grafts were accomplished in identical manner by appositional growth of the host bone. Creeping substitution was only a localized phase in the process of appositional bone growth. Reynolds and Oliver concluded that autogenous bone grafts were superior to homogenous bone grafts only in that the early phase of healing was slightly more rapid and uniform. At the end of 10 weeks no microscopic difference could be seen between the autograft and the homograft. Merthiolate-preserved bone and frozen bone were undistinguishable. Boiled homogenous bone proceeded to union much more slowly.

Herbert and Paillot (91) (1950) pointed out that freezing in air is the least favorable milieu for preserving bone grafts. They claimed that the best results are obtained by immersing the tubes containing the grafts in a liquid (alcohol) mass frozen at −35°. Under these conditions bone cells are intact, with their nuclei. Bone having undergone freezing is identical to bone without freezing.

Extracts of apparently homogenous osseous material from the extremities, scapula, and pelvic girdle of rabbits, freed of muscle and periosteum in the experiments carried out by Roth (92), were preserved in the refrigerator until injected into the extensor muscle of the thigh in adult rabbits. Examinations were made following injection in 41 to 44 days. The extracts of the fresh osseous material gave positive results in a high percentage of instances, i.e., new formation of bone occurred. Extracts of bone preserved at 0°C. for some time gave positive results. The results with bone which had been preserved at −40°C. were less favorable. Extracts of macerated or boiled bone never gave positive results. The maintenance of osteogenetic substances is a decisive factor in bone preservation.

Kreuz and his associates (93) (1951) inlayed cortical grafts in the extensor surface of the mid-shalft of the radius in which a defect had been made. Freeze-dried homografts were incorporated in the same manner as fresh autogenous bone grafts but at a slightly slower rate. The freeze-dried bone was somewhat superior to frozen bone in regard to the rate of healing in the early phases. Except for variations in rate of healing, all the grafts were incorporated in a manner roughly comparable to the healing of the bone defect of the same size in which grafts were not implanted. Kreuz *et al.* believe that freeze-dried homogenous bone is suitable for use in bone banks.

Frozen homogenous cortical grafts stored at −15 to −25°C. were transplanted into the radius of dogs by Marrangoni (94) (1951). Specimens were removed from areas of regeneration at intervals of one to 170 days. All sections were examined microscopically in a decalcified state. Marrangoni considered three factors as influencing the success or failure of frozen homografts: the viability of the transplant at the time of transplantation, the immunologic reaction between the host and graft, and the ability of the transplant to stimulate oteogenesis in the host. He concluded that the fate of frozen homogenous bone transplants is similar to that of autogenous bone transplants except that they are 50 per cent slower in returning to normal consistency. Frozen homogenous bone transplants are not viable but maintain their ability to stimulate osteogenesis.

In a series of experiments on dogs by Wilson (95) (1951) fresh autogenous cortical bone transplanted in muscle incited more reaction and showed greater tendency toward healing than homogenous refrigerated bone pieces. Microscopic examination showed definite osteoblastic activity in the saw cut in the autogenous fragment. There was definite evidence of new bone formation in the autogenous fragment in three weeks, whereas in the refrigerated specimen it appeared only at the end of six weeks. In fresh

autogenous cortical bone transplanted in the ulna, bony callus formation appeared earlier than in frozen homogenous cortical bone. Earlier healing of cancellous bone was noted in comparison with cortical bone when a refrigerated piece of iliac bone was placed in a defect in the ilium. The end-results, however, were the same.

With a modification of Algire and Legallais' transparent chamber technic, Kiehn (96) made observations on fresh and boiled autogenous iliac grafts implanted in the subcutaneous tissues of the rat. The fresh iliac autografts became vascularized within 12 days after transplantation (as seen by the blood vessels growing directly into the graft) and became an integral part of the host tissue. On the other hand, the host tissue reacted to autogenous boiled implant as to a foreign body, as evident in persistent hyperemia and encapsulation of the graft.

When fresh iliac chips removed from a dog were placed immediately in the abdominal subcutaneous tissues of another dog, Kiehn noted that radioactive phosphorus (P^{32}) appeared to be taken up at a slower rate in homogenous grafts than in autogenous grafts.

In the experiments by Ham and Gordon (97) (1952) cancellous bone chips were obtained from the crest of the ilium of dogs. One half of the autogenous bone chips, thrice frozen in carbon dioxide ice for 10 minutes and thawed in warm saline, and the other half untreated, were transplanted into the muscles on the other side as autografts. Sections of pieces of muscle were examined at periods of 5, 7, 21 and 28 days after operation. In none of the dogs did new bone form in association with thrice frozen and thawed chips. Ham concluded that the new bone that formed around untreated autogenous cancellous chips arose from such covering cells of the chips, or undifferentiated marrow cells associated with them, as survive transplantation.

Working with rats, Karcher (98) in 1953 found that P^{32} consumption during his whole period of observation was less in homotransplants than in autotransplants. Grafts preserved at 0°C. and implanted under the skin or in bone absorbed less P^{32} than fresh transplants in similar trnsplantation sites. Preserved autogenous and homogenous grafts absorbed less of the isotope than fresh autogenous and homogenous transplants. The duration of preservation had no essential influence on the absorption of the isotope.

To determine the value to accord preservation of bone, Sicard and Mouly (99) made an experimental study of the histological, physical and biochemical qualities of autografts and preserved homografts. A resected femoral fragment from the diaphysis, preserved by refrigeration, was placed some days later in the femur of another dog. They concluded that the union of the autogenous fresh grafts and that of preserved homografts was by identical processes. The fresh autografts unite much more quickly than the preserved homograft, their appearance being very different on the fifteenth day. The graft, either fresh autogenous or preserved homogenous, always unites completely at the end of about two months. The osseous reconstruction maintains a metamorphosis of fundamental substance, probably polymerization of the glycoproteins, which constitutes it and which is itself bound to a secretion of mesenchymal cells. This led Sicard and Mouly to the thought that osteogenesis, which is the act of the graft, is bound to it by an unknown factor. Whether it is a matter of a supply of phosphatase is impossible to verify at this time.

Using the transparent chamber method, Williams (100) studied the survivability of 20 different tissues as autografts and 15 as homografts in 110 rabbits. The surviving tissues were studied for not less than 8 months. Autografts were able to stimulate the endothelium transplanted with them

and also that of the vessels in the host region. The graft determined the nature of its vasculature, not the vessels on which is was placed. Homografts generally had little ability to stimulate endothelium, but if they did, the vessels could not be maintained. The structure and organization of surviving grafts tended to duplicate that of the whole organs from which they were obtained. Grafts, if they survive, create their own intercellular environment. Failure of a graft to survive may be due to inability of the transplanted cells to function adequately and thus replace their protective intercellular substance rather than to immediate attack by any defense mechanism against foreign cells in the host region. Williams noted no correlation between graft survival and white cell infiltration.

HETEROPLASTIC BONE GRAFTING IN ANIMALS

Being a versatile investigator, Ollier (101) (1867) also observed that heterografts from inferior to superior species were no more successful than from superior to inferior species. He found that heterogenous periosteal transplants formed only a fibrous membrane.

In 1919 Gallie and Robertson (28) reported on experimental work with heterogenous bone grafts from cats to dogs, in contact with muscle and bone. At the end of 8 weeks circulation was well established and the cells had completely disappeared. Bone was surrounded by fibrous tissue, with occasional giant cells resting on the surface in lacunae, indicating some slight degree of absorption. No osteoblasts and no formation of new bone were observed. Heterogenous bone was considered to be useless for transplantation.

In experiments on dogs, Davis and Christopher (102) (1924) found that intramedullary pegs of boiled beef bone in contact with the endosteum of host bone were surrounded by living bone and became solidly embedded in new bone. They were later absorbed and replaced by living bone. Beef bone not in contact with endosteum was absorbed rapidly but was not replaced.

Autogenous bone grafts and homogenous bone grafts, fresh and preserved in Ringer's solution for 4 to 10 days, as well as heterogenous grafts were transplanted by Gaudioso (103) (1924) with good results. He believed it possible for the anatomical and functional restitution to be completed, as the presence of a graft stimulates the host tissues to osteogenic repair. In one instance of human bone transplanted in the femur of a rabbit he noted microscopically that the graft gradually decomposed and young connective tissue began to take its place. In the proximity of the graft new osteoblastic formation was observed as coming from the host periosteum.

In the experiments carried out by Finaly (104), of St. Josephe's Hospital, Heerlen, living human bone sections were tied together immediately after removal with strands of sterilized woman's hair and placed in the thigh muscle of a rabbit. After 3 weeks the specimens were examined macroscopically and microscopically. The bone fragments were grown together and united by callus. Thus, they evidently live following transplantation, since callus formation could not have taken place otherwise. The fact, Finaly concluded, that periosteal union developed definitely proved that the graft had survived, and that the duration of survival sufficed for complete repair of an osseous defect at a given site.

The question arose whether this continued vitality springs from the graft itself or whether the surrounding tissue plays a significant rôle. For the formation of a connective-tissue capsule, a certain time interval is required during which juices from nutrient media can reach the transplant. If the capsule formed is not wholly impermeable to fluids, it must be assumed that any graft makes immediate use of nutrient from the

recipient. Its further survival will then depend on the degree to which it can assimilate and utilize this nutrient. Finaly further concluded that survival of the bone fragment and its fusion result from active metabolism, with the transplant assuming a parasitic character.

That the callus formed consisted of atypical periosteal connective tissue could be explained by the fact that the character and durability of regenerative new formations in grafts seem to depend largely on the function of the transplanted tissue. In the experiments reported here the bone tissue was entirely devoid of function.

In the Research Department of Northwestern University Medical School, corticomedullary fixation of the humeral fracture in 7 dogs with cow's horn was carried out by Fowler (105) in 1934. Corticomedullary horn splint was inserted into the fractured ulna in 4 dogs. In 2 dogs which were killed, an appreciable amount of horn was absorbed in 4 weeks, and fully half of the horn in 3 months.

Calvé (106) in 1935 replaced the resected fibula (with its periosteum) in rabbits with spongiose calf tissue preserved in ether. The two ends of the fibula were reunited by a bridge of soft grayish substance adherent to the tibia. Active osteogenesis was noted in histologic examination, the transplants of dead bone behaving exactly as those of living bone.

Liberti (107) in 1940 reported a series of experiments in which segments of the radii of rabbits were removed. The defect was filled with ox bone soaked in various solutions. Only those immersed in 2 per cent cholesterol in oil, or in equal parts of cholesterol in oil and rabbit's blood showed regeneration. He believed that ox bone treated in such a manner could be used in place of autogenous bone.

Stark (108) (1942) carried out a series of experiments on rabbits. Os purum and os novum (see Orell, page 206) were transplanted subperiosteally into a defect in the forelegs of rabbits. The defect was healed with os purum in six weeks and the implant might remain visible on roentgenograms for three years. At six weeks the autogenous graft was healed and blended. Os novum healed more rapidly than autogenous transplants but the necessity for a second operation increased the risk.

In the experiments reported by Hughes (109) (1943) portions of the femur and radius were excised in dogs, and of the fibula in rabbits, and cylindrical pegs of ivory, cow horn, and beef bone (boiled in some instances) were inserted into the bony defects. In long bones foreign substances, as summarized by Hughes, located within bone cortex are absorbed slowly. Substances within the medullary cavity are absorbed more rapidly, and materials which are extracortical in position are absorbed most rapidly. More callus was formed around a beef bone peg and still more callus around ivory or cow horn than around an autogenous peg. No firm union was formed between ivory or cow-horn pegs and the host bone. Microscopically it appears that there is definite bony union between the autogenous or beef-bone pegs and the host bone. Beef bone is a much better substitute for autogenous bone grafts than ivory or cow horn.

Examining microscopic sections of refrigerated fetal bone, Le Cocq and his colleagues (110) found the staining reaction was the same as that of fresh bone, with the cellular pattern assuming normal characteristics. This observation indicates that bone tissue remains in the fresh state during preservation. Transplantations in a guinea pig verified that cells in the bone transplant failed to survive. It would appear that success of the preserved bone depends on its affinity for revascularization.

Guilleminet, Stagnara and Dubost-Perret (111) (1950) used bone, compact or spon-

giose, in onlay or inlay, removed by veterinaries with sterile precautions and exposed to ultraviolet radiation, then preserved in two tubes, one within the other, at −70° until the time of use. In the first series they transplanted bone from the sheep to the dog's radius, followed by radiographic examination in 30 days, and biopsies in 60 days. In the second series a bone defect in the dog's radius was replaced by refrigerated compact bone from the sheep. Osteosynthesis of a femur of the dog, fractured accidentally and fixed operatively by a plaque of refrigerated compact bone from the sheep, was produced, with good consolidation in 5 months. Guilleminet and his colleagues concluded that refrigerated heterogenous bone transplants seem to behave as autogenous grafts from the radiologic and histologic viewpoints. Heterogenous bone transplants not refrigerated appeared to behave usually as aseptic foreign bodies and were resorbed to a more or less degree. The results were better when the bone grafts were placed in an osseous cut than on the peristeal denuded surface of a diaphysis.

A film illustrating the work on animal osseous grafts at the Clinique Chirurgicale Orthopédique et Infantile de Lyon (112) (under Guilleminet) was reported in 1952. The bone bank where grafts are furnished by removing the diaphysis of the calf is seen. There are views of the locations permitting selection of the animal, (abattoir in optimal condition), steps in removing bone, with guarantees of asepsis under ultraviolet radiation and numerous bacteriologic controls. The grafts are transported in a refrigerator at −40°C.

The experimental review begins with a coverage of the work of Ollier. Experience with transplantation of calf bone to the dog is presented. Histologic documents show the quality of assimilation of refrigerated bone transplanted to a different species.

SUMMARY OF EXPERIMENTAL WORK ON ANIMAL GRAFTS

The great volume of experimental wor with bone grafts in animals (only a moderat portion of which is reviewed in this chapte is in part somewhat boring and certainl confusing. It appears necessary, however, t enumerate the more important works as form of good scientific manners and *as an im portant background for future experiment work*. Many of the present-day contributior on bone grafts will probably be just as te dious to our successors in this interestin field.

As one reviews the findings in the exper mental work on bone grafts in animals, th first impression is confusing because of th apparently conflicting data. Different inte pretations and theories are often include with the factual part of the work, which add to the perplexity of the reviewer. Exper ments with bone transplants in such idea locations as the anterior chamber of the ey and in tissue cultures are most difficult t interpret in respect to the relationship c the findings with the behavior of bone graft transplanted in contact with bone or in so tissues in a living animal. If we follow th scientific method of approach, however, an separate facts from interpretation of facts some order begins to emerge.

Factual Data

That the facts regarding the behavior c free bone grafts in animals are often con flicting may be owing to the different exper mental animals used. Bone grafts in rabbit may behave differently from similar graft in dogs, guinea pigs, cats etc.

Furthermore, the donor sites of the bon grafts were various localities in the variou animals, and the investigators seem to hav assumed that bone is bone wherever it come from and should behave in the same wa This is not borne out in the human exper mental work in which the author observe

that free bone grafts from the nasal bone, septum, and turbinate survived as bone after transplantation in fat, whereas rib, tibial and ilial grafts buried in fat were replaced by fibrous tissue. There are no reports on the burial of nasal, septal and turbinate bones in animals.

In the older literature little distinction is made between dense cortical bone grafts and the more open cancellous bone grafts. Most of the older experimenters, however, were influenced by the pioneer work of Ollier and they always emphasized the presence or absence of periosteum on their grafts, an item which some later workers have failed to observe.

Another difficulty confronting a reviewer is the length of time elapsing between burial and removal of the graft by different experimenters in different animals. In the human also, it is known that some simple onlay grafts in contact with bone but not serving any function of stress and strain, tend to become reduced in size after forming osseous union with the host bone. Is this a consistent factor in animal grafts and is it related to the normal life span of the animal?

All these difficulties notwithstanding, a digest of the facts in the articles reviewed in this chapter and of many additional articles not included leads to the following conclusions.

On the basis of experimental observation the majority of earlier writers agree that osteogenesis is chiefly executed or controlled by osteoblasts in the deep layer of the periosteum (Ollier). Osteoblasts in the endosteum and haversian canals also play a part in new bone formation. The bone cells themselves, enclosed in a rigid calcified matrix which renders them incapable of initiating interstitial growth, do not take any part or an active part in osteogenesis.

Leriche and Policard believe that bone formation does not occur through the agency of osteoblasts. Instead, osteogenesis takes place through the activity of undifferentiated connective-tissue cells and may occur anywhere in the body. The majority of later investigators subscribed to both theories of bone formation (Ollier, and Leriche and Policard), believing that osteoblasts in the periosteum, endosteum, and haversian canals as well as undifferentiated mesenchymal tissue have osteogenetic powers.

Levander and others have proposed the presence of a substance liberated from a bone graft which serves as an activator and induces osteogenesis in the surrounding mesenchymal tissue. These investigators believe that osteoblasts in the periosteum are important for bone growth, the healing of fractures and the fixation of bone grafts until the bones are fully developed. In adult life osteogenesis is affected mainly by undifferentiated mesenchymal cells.

Most recent investigators agree that bone grafts retain their calcified structure best when they are transplanted in contact with bone and immobilized until firm union between graft and host bone has taken place. Grafts take equally well whether transplanted with or without periosteum according to some, while others believe that grafts with attached periosteum do better.

Factors in normal function such as stress and strain tend to help healed bone grafts to retain their calcified matrix according to most animal investigations. Later observers in general agree that healing is more rapid in fresh autogenous bone grafts and more delayed in fresh, preserved and frozen homogenous bone grafts. Some believe that cancellous bone heals more quickly than cortical bone.

Most investigators believe that *all or most of the osteocytes in a free autogenous bone graft degenerate and die following transplantation*, but a few experimenters demonstrated that some of the bone cells in the graft survive and participate in new bone formation. *The cells in fresh homogenous bone grafts always*

die and are replaced by new cells from the host tissue.

The belief is prevalent that all bone grafts in contact with bone are gradually absorbed and replaced by some sort of creeping substitution from the host bone and from osteoblasts in its periosteum. One infers that this new-bone infiltration from the host bone is through the agency of osteoblasts in its haversian systems and marrow cavity. Some investigators have observed, however, that autogenous bone grafts with periosteum when transplanted in soft tissue not only retain their calcified structure but give evidence of new bone formation. Autogenous periosteum transplanted in soft tissue has produced bone for some men but has failed to produce bone for others. It is noteworthy to recall that Ollier himself did not always find bone formation in his periosteal grafts. He emphasized the instances in which bone formation did occur, and when it was absent he believed that he had failed to include the deep cambium layer with his periosteal graft.

Histologic Changes Following Bone Grafting

These changes in animals following auto- and homotransplantation are fairly clear. A fibrinous exudate causes the surrounding host tissue to unite with the graft, and as this becomes organized, collagenous fibers are formed through activity of certain fibroblasts which are osteoblasts or mesenchymal cells. Callus formation occurs as a deposition of mineral salts in the collagenous mesh. This effects a union of the graft with its host bone as in a healing fracture, but degeneration of bone cells in the graft and replacement by creeping substitution begin quite early, as does vascularization of the graft.

Absorption of the bone graft takes place from the marrow cavity, the haversian canals and from the surface so that the entire graft becomes quite porous. The bone replacement occurs simultaneously with the degenerative phase so that the new-bone formation keeps pace with the absorption of dead bone. Thus, the architecture of the graft is maintained until the graft is completely or (in autogenous grafts) almost completely replaced by living bone. In autogenous grafts possibly a few osteocytes and osteoblasts in the graft survive and participate in the new bone formation.

The osteogenic cells in bone at fracture sites possess the capacity to form either cartilage or bone. According to Ham (113) the vascularity of their environment determines whether cartilage or bone is produced. If the area is rich in capillaries, the osteogenic cells differentiate into osteoblasts and form bone; in avascular areas the osteogenic cells differentiate into chondroblasts and so form cartilage. The external callus, when it has grown sufficiently to have united the two fragments, usually consists of bone, cartilage, and osteogenic cells. The cartilage later becomes calcified and the chondrocytes die from lack of nutrition. Osteogenic cells accompanied by capillaries invade the cartilage and replace it with bone.

Many investigators believe that certain cells with the abilities of undifferentiated mesenchymal cells persist in adult tissues. They are said to be smaller than fibroblasts but have the same general appearance. The conviction that these persistent cells are not common fibroblasts but are undifferentiated cells is based on numerous observations that under the influence of certain stimuli they may furnish new cell types (114). Some investigators believe that under the influence of external stimuli all fibroblasts can furnish new cell types. *New bone and cartilage formaation at fracture sites undoubtedly arises in part from the activity of these undifferentiated connective-tissue cells or from ordinary fibroblasts which revert to this cell type.*

Of particular interest to orthopedic surgeons is the experimental work on animals involving the subperiosteal transplantation of entire long bones with their articular

cartilage surfaces. Hans May (115) and other investigators have removed long bones subperiosteally and retransplanted them as free autogenous grafts in their original site. May noted that the entire graft became vascularized but that the osteocytes in the graft died. Later on the dead bone was transformed into living bone tissue by osteoblasts which accompany the ingrowing vessels, but only if the graft is protected by periosteum. If it is denuded of periosteum the graft becomes destroyed by the ingrowing fibrous tissue. The periosteum, according to May, is the only reliable factor in regeneration when an entire bone, with its medullary cavity closed, is transplanted

Although many authors refer to heterogenous bone transplants as being inferior to autogenous and homogenous transplants, there is a scarcity of publications dealing with the actual burial and removal of bone heterotransplants in animals. Gallie and Robertson transplanted heterogenous cat bone into the muscle and bone of dogs. They found the grafts to be unsatisfactory for transplantation; no viable cells survived and the dead bone was completely absorbed. New-bone formation did occur from the host bone but the process was delayed. Experimental evidence indicates that homografts are more dependable than heterografts.

In general, the majority of investigators agree that autogenous bone is the best graft to use, since healing is more rapid. Homogenous bone grafts, either preserved or stored fresh at refrigerator temperature or placed in the deep freeze, are also useful.

A number of men believe that fresh homogenous grafts placed in the deep freeze are superior to homogenous grafts stored in preservatives such as merthiolate and alcohol, or grafts which have been subjected to heat. These investigators claim that the cells in the quickly-frozen grafts survive, and that the presence of living cells in homogenous grafts is advantageous for earlier and more complete replacement of the graft by the host bone.

It is true that the cells in certain lower forms such as the amphibia and fishes can be frozen for periods of time and still regain their viability when thawed out. There are authentic reports of the survival of the entire organism after freezing. Thus frozen frogs and salamanders revive and move away, and fish which have been caught and thrown on ice so that they are frozen, revive when the startled fisherman thaws them out in warm water preparatory to cleaning. This ability to withstand freezing, however, has not been demonstrated in the higher mammals as actual revival of the complete organism.

Regarding the interesting studies of the viability of autogenous frozen bone grafts in animals, one should note that the taking up of radioactive phosphorus (P^{32}) by the bone is not necessarily evidence of metabolism or cellular viability. It is suggestive rather than conclusive and like so much experimental work induces the investigator to do further work to prove what he thought had been demonstrated.

Growth of cells is certainly positive evidence of viability, and one would think that the tissue culture method applied to the cellular elements in grafts would be the most direct way to settle the matter.

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CHAPTER 17

Autogenous Bone Transplants in Humans

It would be most gratifying to write about the exact behavior of autogenous bone grafts in humans with assurance and decision. Unfortunately this cannot be done with the same degree of confidence as one feels in describing the behavior of autogenous cartilage grafts.

For instance, autogenous bone grafts from the ribs, ilium, and tibia lose their calcified matrix and disappear when buried in soft tissues. When these same grafts are buried in contact with living bone and sufficiently immobilized they retain their calcified matrix. Autogenous cartilage grafts do equally well whether buried in contact with cartilage or in soft tissues.

All types of autogenous cartilage grafts behave in about the same way but some types of bone grafts survive transplantation, whereas other types in similar recipient sites lose their calcified matrix and are replaced by fibrous tissue.

The healing process in human cartilage is quite simple, since it does not exist insofar as cartilage regeneration or replacement is concerned. In bone the healing process by new bone formation occurs with consistent regularity, but the numerous factors initiating and controlling it are somewhat speculative, to say the least.

Do special cells, the osteoblasts, have a monopoly on the process of new bone formation like the melanoblasts for melanin pigment and the fibroblasts for the production of collagenous fibers? Do these osteoblasts have a specific and fixed abode in the cambium layer of the periosteum, haversian canals, and endosteum, or do the mesenchymal cells from which they originate have a wide distribution in all soft tissues?

Certainly osteoblasts are present where new bone is being formed. Do these cells have an active part in the process, or is osteogenesis a sort of chemical process like the precipitation of salts in a test tube?

What is the cellular agency for the absorption of bone? Rib, tibial and iliac bone grafts undergoing absorption in soft tissue sites have numerous fibroblasts and blood vessels in the area where bone is being absorbed. Do the fibroblasts play an active role in this absorption?

One also speculates about the function of the osteocyte, which is present in all bone and, presumably, is the parenchymal cell of bony tissue. It survives free transplantation in septal, nasal and turbinate bone grafts and is associated with, or actively maintains, its calcified matrix. When the osteocytes in autogenous septal, nasal and turbinate bone grafts are killed by heat before the graft is transplanted, the calcified matrix is slowly absorbed. The presence of living osteocytes, therefore, is essential for the retention of the

calcified matrix, which in the absence of living osteocytes is absorbed and replaced by fibrous tissue.

Bone grafts and their behavior after free transplantation compel respect. In writing about this interesting tissue one must be extremely careful to differentiate between established facts and the numerous attractive theories advanced to explain these facts. It is wise also to accept the results of animal experimental work as suggestive rather than conclusive regarding the fate of bone grafts in humans. This applies particularly to animal experiments with bone grafts in such ideal locations as the anterior chamber of the eye and to the survival and behavior of bone in tissue culture.

Experimental work with bone grafts in humans has consisted largely of clinical examination of the grafted area, radiographic studies of the transplant and its adjacent host bone, and occasional microscopic study of a graft which was unsatisfactory to the patient.

It is not easy to bury bone grafts in humans and remove them at selected intervals because patients have an understandable disinclination to be experimented upon, and there is always the chilling thought of a possible law suit.

The author has been most fortunate in obtaining the cooperation of many patients having operations in numerous stages, so a relatively large number of bone grafts have been transplanted and removed with full understanding and permission of the patients. In this way 67 autogenous bone grafts, both with and without periosteum, were transplanted in soft tissue sites and removed for microscopic examination at selected intervals. Twenty autogenous rib, tibial and iliac bone grafts were transplanted in contact with bone and later removed and examined.

The findings in this experimental series of human autogenous bone grafts naturally influence the attitude of the author in describing the behavior of bone grafts. It is interesting that so much of the factual evidence in this series supports rather than contradicts established beliefs. Perhaps the most important new finding in these experiments was the fact that *septal, nasal and turbinate bone grafts remain as bone* after transplantation in soft tissues and that the *osteocytes or bone cells survive as living cells*. This rather definitely contradicts the established belief that the bone cells in human autogenous bone grafts always die and are replaced by living cells from host bone.[1]

REVIEW OF LITERATURE ON AUTOGENOUS BONE TRANSPLANTS

Bone grafting was probably practiced on man as a clinical procedure about a century ago. It may be said, however, that the practice of bone transplantation as an approved clinical procedure belongs to modern times. The main impetus to the employment of grafting was given by John Hunter (1) (1728–1793), but the grafting of bone, as viewed by Ollier (2) in 1858, was a dangerous procedure in humans. Ollier, it may be recalled, was the father of bone physiology, based on animal experiments.

As we study the subject it becomes evident that although there has been much change in views and theories regarding the application of bone grafts in late years, still a great deal remains unknown regarding the behavior of this perplexing tissue.

Early Investigations

In 1875 Nussbau (3) recommended rotating a fragment still attached to the lower end

[1] Recent unpublished experimental work by the author demonstrated that complete finger and toe bones with joint capsules, epiphyseal cartilages, and nails retain their calcified structure when buried as autografts in abdominal fat up until twenty-two months. From a gross standpoint, the bones are freely movable and the nail bed appears to have survived. The transplants were made in infants and children with supernumerary fingers, and the periosteum was left on the bones.

so as to bridge a defect in the ulna, and fastened across a 2-inch defect in about the same way as the slide graft of present-day use. In 1886 Bircher (4), of the University of Berne, found the inlay of intramedullary ivory pegs to be the simplest method of retention in fracture of the long bones. He also suggested the locking of fracture fragments with an I-shaped graft and reported good healing and consolidation in five cases of simple fracture.

Adamkiewicz (5) in 1889 came to the conclusion that small autogenous pieces of bone would reunite if they were replaced after removal by trephining. He expressed the idea that osseous structures should come in contact but that they need not fit accurately. He believed that the periosteum took no active part in the process of repair but that the cellular connective tissue which formed underwent ossification. He mentioned growth of bone proceeding from the periphery surrounding such bone transplants. In a case of ununited fracture of the tibia reported by Curtis (6) (1892) there was destruction of bone with loss of substance. A segment of the patient's fibula was cut out and pushed through the soft tissue into the gap in the tibia. Firm union took place; weight-bearing on the limb was possible in two months after operation. This was followed by four additional successful cases.

In absence of the humerus after acute osteomyelitis, Bardenheuer (7) (1896) transplanted an autograft of the spina scapula; firm union resulted. In another case an autogenous transplant of the spina scapula was placed in a defect from resection of a tuberculous shoulder joint, resulting in good function.

Murphy (8) in 1899 implanted the second phalanx from a patient with dactylism, in the nose as an autogenous graft. All the periosteum and both of the cartilaginous ends were retained on the graft. In 14 months the entire transplant disappeared. In 1901 he grafted the hypothenar eminence of the hand to the nasal bone, including the fifth metacarpal bone, by fixation of the hand to the head and noted that it retained its viability.

Delayed callus formation relative to beginning pseudarthrosis was observed by Bier (9) in 1905. He regarded blood extravasation as the natural stimulus for callus; in addition, through excitation of inflammation, it brings about increased nourishment. Extravasation is a nutrient medium directly and indirectly because young cells of the callus probably consume it and apply it to bony structure. Extravasation, in his opinion, is necessary for healing of bone fracture.

Lexer (10) (1908) decided that when autogenous bone grafts are transplanted with periosteum and endosteum the transplanted bone always dies but the periosteum and endosteum survive. The dead bone is later regenerated from the surviving periosteum and endosteum.

Vorschütz (11) (1911) transferred an autogenous free graft from the right tibia, covered with periosteum, to the region of the lower jaw which had been removed because of malignant tumor. After formation of a fistula and sequestration, healing occurred in the lower part. In another case in which the lower jaw had been resected because of actinomycosis, an autogenous piece from the tibia was transplanted. After five to six months bone could be palpated. Excellent cosmetic and functional results were obtained in two cases.

Carter (12) (1911) transplanted autogenous rib, with the periosteum removed, into the nose in 9 patients to repair saddle deformities. The medullary tissue was scraped from the outer half of the graft, and the skin and subcutaneous tissue over the dorsum and sides of the nose were elevated, as well as the periosteum over the nasofrontal process. The strip of bone was inserted into the wound in the nose, reaching nearly to the tip, and the upper part anchored under the

periosteum over the nasofrontal process. There was no sign of the disappearance of the graft or any irritation at the end of 4 to 18 months.

Hibbs (13) (1911), who was a master technician, achieved a remarkably high percentage of good results in his bone grafting. After denuding the laminae of cortical bone and removing all cartilage from the intervertebral joints, he used the spinous processes and the tiny bits of bone supplied by the denudation to fuse a diseased spine. He applied his method, with slight modifications, to fuse the hip, the knee, and other joints.

In 1912 Murphy (14) reported on his observations and analysis of microscopic photographs and pathologic specimens, clinical and experimental, by others. Bone, with its periosteum, transplanted in soft tissue in the same individual and free from bony contact, practically always dies and is absorbed. Autogenous bone transplants, with or without periosteum, in contact with living bone become united to living fragments and act as a scaffolding for reproduction of new bone of the same size and shape as the transplanted bone. The rôle of the transplanted fragment is to give mechanical support to capillaries and blood vessels, with their living osteogenetic cells, as they advance from the living bone at both ends of the implanted fragment into the haversian canals, canaliculi, and lacunae of the transplant. Ultimately all of the transplant disappears; new lamellae are formed by the osteoblasts, and the graft lamellae are removed by the osteoclasts. He concluded that the graft is *per se* not osteogenetic but osteoconductive. The regenerative forces and cells are entirely supplied from the osteogenetic cells which are nourished by the capillaries growing from living bone. The graft, however, is an absolute necessity in the regeneration. Periosteum attached to the transplanted portion when taken from the young has a "plus osteogenetic influence"; in the middle aged it is neutral, and in those of advanced years it plays a minor rôle and, in fact, is detrimental.

McWilliams (15) (1912) transplanted a piece of autogenous rib, subperiosteally resected and separated from periosteum, into a lower-jaw defect resulting from the removal of giant-cell sarcoma; it was successful after two months, healing by primary union. In another patient, autogenous rib was transplanted into the tissues of the inguinal hernia to strengthen the canal.[2] Primary union also occurred. He had the idea of shaving bone in small pieces for transplantation, and he considered periosteum as having a very important function in maintaining the nutrition of the graft. In several of his cases where rib bone was stripped of periosteum and implanted in bony contact or in soft tissue, the graft disappeared. Clinically, when bone (healthy fibula) with periosteal covering was implanted, new bone formed along the periosteum of the graft. McWilliams believed that the callus arose from the periosteum or from the bone of the graft itself. To be assured of subsequent living of a bone graft, it should be transplanted with as much periosteum covering its surfaces as possible (16).

The clinical observations made by Phemister (17) (1915) conformed to a certain degree with his experimental results (see Chapter 16, page 155). Subperiosteal resection of a non-infected shaft in young persons is followed by regeneration from the remaining periosteum and not by outgrowths from the ends of the bone.

Gallie and Robertson (18) (1919) held that from a clinical standpoint the periosteum is of great importance because of its

[2] Diced cartilage or shavings, whether autogenous or from a bank, serve very well to strengthen the fascial support in inguinal hernia. Autogenous rib bone in soft tissue sites is absorbed and replaced by fibrous tissue.

control of the circulation throughout living bone. Extensive stripping of the periosteum at operation will result in necrosis, which may cause delay in union and will result in sequestration where sepsis is present.

Albee, who wrote the first book published in any language on the sole subject of bone graft surgery (19), was a pioneer in introducing a method of bone grafting for Pott's disease of the spine. He believed that a bone graft always acts as a stimulus to osteogenesis in the bone into which it is engrafted or with which it is in contact (20). Survival is enhanced by exact approximation of the components of the graft with the same components of the host bone. He was convinced that the best transplant is a live piece of autogenous bone including periosteum, complete thickness of the cortex, endosteum, and marrow. Transplanted living bone tissue becomes a part of the osseous system wherever it has been implanted. Albee also used "sliver grafts" alongside the main fixation graft to furnish additional foci of bone growth (21).

Neuhof (22) in 1923 expressed the belief that the liberation of substances from the slowly-disintegrating graft constitutes a physicochemical stimulus, resulting in the metaplastic formation of bone tissue from the adjacent host connective tissue. He further stated that the bone cells of the graft die more or less rapidly, depending upon their distance from the source of nourishment in the host, and the periosteum and endosteum undergo at least partial necrosis. However, primary adhesion to the host tissues occurs most readily in the presence of periosteum.

Lewin (23) (1924) repaired a large fragmented wound in the left side of the skull with autogenous bone, including periosteal covering, from the fifth and sixth ribs of the same side. The margins of the ribs were "rawed" and the ends trimmed; the pericranium was stitched over the ribs, and the flap of the scalp replaced. The patient made an uninterrupted recovery, the cranial defect becoming filled in by a regular sheet of bone.

Báron (24) (1926) successfully used the astralgus as the source of the transplant in operation on a child with pseudarthrosis following fracture of the shaft of both bones of the left leg about four years previously. The conical ends of the tibial fragments were wedged in the transplant and the whole covered with a strip of periosteum removed from the other tibia. He held that spongy bone, combined with periosteum, or with periosteum and cortex, is the best material for bone transplants.

Later Investigations

Matwejew (25) (1930, from the Kasan-Levin Institute) used bone plate from the tibia that had been implanted under the skin of the left shoulder, to repair a nasal defect in a 35-year-old man. Histologically, the implant showed bone and cartilage tissue, and permeation with bone cells interlaced with numerous haversian canals. The new bone was thought to be differentiated in structure and character from the implanted bone.

Carter (26) (1930) considered the rib, with its outer layer of periosteum, as best suited for bone transplantation. He used combined bone and cartilage transplant in repairing saddle-nose deformity in which the nose is too short, the cartilage end of the implant being introduced as far as the tip of the nose without destroying its resiliency. In the case reported there was increased growth of the transplant at points of contact with the frontal and nasal bones two years after operation.

In a case of myxochondroma metacarpi reported by Petrow (27) (1933), a proximal phalanx of the big toe from the left foot was used for the defect produced by resection along with the whole proximal part of the bone. There was complete change in struc-

ture of the transplant 25 years and 9 months after the operation. The inner bony prominence remained, while the external one was absorbed.

Orell (28) in 1937 expressed his belief that boiled fresh bone often has the proper shape and structure for replantation when bone is in a pathologic condition and other types of osseous grafts are not so applicable. In some instances he resected the diseased bone, boiled it, cleared it of diseased tissue masses and replanted it for mechanical support until new bone developed. Such boiled autogenous bone serves as a stimulant to living connective tissue in the bed. Boiled bone contains fat, connective tissue and proteins. According to Orell, when boiled and dried bone is used for grafting, its resorption and the growth of new bone take place very slowly and much less satisfactorily than when boiled fresh bone is used.

In the opinion of Esmaurrizar (29) (1940) of Mexico City, some transplanted living cells in living bone transplants may possibly continue to develop, but most of them disappear and are replaced by new cells derived from the surrounding tissues. He thought that the grafts have mechanical functions (sustaining and refilling), chemical functions (as a source of mineral salts *in situ*), morphological functions (the bone canals serving as guides to the medullary cells, promoting the formation of new bone within the graft), and perhaps biological function, stimulating osteogenesis.

In a roentgenographic and biopsy study of autogenous human iliac bone grafts by Rainsford Mowlem (30) in 1941, the survival of the bone cells and calcified structure of iliac bone grafts appeared to depend upon the nourishment which the graft receives after transfer rather than upon its contact with living bone. The cells in cancellous bone grafts will survive as living cells associated with calcified matrix when the graft is transplanted into a vascular bed whether or not it is in contact with bone. When cancellous iliac bone grafts are transplanted into an avascular bed, the cells in the graft die and the graft structure is replaced by fibrous tissue. It is Mowlem's belief that *the cells in dense cortical bone grafts tend to die*, even when they are transplanted into vascular beds, because of lack of early nutrition. Mowlem noted that iliac bone grafts not in contact with bone retained their calcified structure up to periods of one year but all of these grafts *were transplanted into the nose*; none were in soft tissue elsewhere, either vascular or avascular. Thus Mowlem's factual observations confirmed Carter's conclusions that the soft tissues of the nose constitute a rather favorable transplantation site for bone grafts. At any rate the site is favorable for the survival of iliac and rib bone grafts (30).

Fragmentation of cancellous bone makes a greater proportion of its cells accessible to the blood supply and expedites its survival (Mowlem) (31).

Blocker (32) (1946) emphasized that autogenous tissues are generally used in the fresh form. Many congenital deformities and defects following trauma have been repaired by autogenous bone transplants, but not as successfully as had been hoped. The fact that a quantity of bone is accessible and is able to withstand strain has led to its popularity as a material for grafting.

What happens grossly following bone transplantation in contact with bone was described by Blocker as follows:

Healing following transplantation of bone occurs in much the same way as after primary fracture. A clot is organized in the graft bed and organizing vascular connective tissue invades at a rate which is in reverse proportion to the density of the graft. After the establishment of vascularization some bony absorption takes place, and finally calcium is redeposited and firm bony union results.

Most investigators believe that the graft is slowly replaced by a process of creeping substitution from elements in the host bone or from its periosteum; but others, including the author, believe that the bone cells under favorable conditions survive *en masse* and retain the calcified structure of the graft.

Compact bone grafts are as a rule obtained from the crest of the tibia, while those of cancellous bone generally are taken from the crest of the ilium. Massive bone grafts, in particular compact bone, generally die and serve only as a trellis for the formation of new bone (33, 34).

In a series of cases Flanagan and Burem (35) obtained union of defects in the tibia and femur by apposing massive grafts, with supporting external cortex in the form of a cylinder and reestablishment of continuity of the medullary canal. The massive grafts become integral parts of each other as well as of the host bone itself, depending upon adequate blood supply to the grafts and positive fixation of the grafts internally.

Recent Literature

Horwitz (36) (1949) advocated the use of cancellous bone as a graft to bridge or fill an osseous defect at the end of the long bone, where spongy bone normally predominates. Cancellous bone containing red marrow appears to have greater osteogenic properties than cancellous bone in which yellow marrow predominates. The iliac wings consist of an enormous quantity of red marrow.

In a case reported by Catalona (37) (1951), a compound fracture of the middle third of the left leg had been treated elsewhere. Severe infection occurred, followed by drainage. When the patient was seen, the leg was flail in the middle third, with dense scarring of the skin and so forth. In a Flanagan-Burem operation the scars were excised, and thick split-thickness grafts taken from the abdomen were applied. Three months later, an apposing hemicylindrical massive bone graft was used in reconstruction, following which osteogenesis and union were observed. At the end of a year the grafts were becoming an integral part of each other as well as of the host bone itself. The patient returned to work, without using a cane. Catalona pointed out that the intactness of the periosteum, the necessity of interfering with intermediary callus and sclerosing bone ends, the need of internal fixation, the use of inlay, onlay, single or dual grafts and of cortical or cancellous bone graft, and the presence of infection, scar tissue and unhealthy skin should be carefully considered if healing is to be given an optimum chance to occur (Abbott, Schottstaedt, Albee and many others).

Levander had concluded that beside the osseous tissue the periosteum and marrow implanted with it die off entirely in transplantation. Axhausen (38) (1951) does not regard the investigations by Levander as sufficing to overthrow the histologic laws which lie at the foundation of the classic osteoblastic theory. Many pathologic bone findings, as stated by Axhausen, tend to show that in the death of bone a stronger stimulation to bone formation comes from the periosteum belonging to it. This stimulation is exerted forcefully, in particular on the mesenchymal tissue. The observations on transplants of autogenous bone covered with periosteum agree with this finding. Autogenous bone covered with periosteum must maintain its commanding position in repairing large defects of the tubular bones and lower jaw, for the forces of osseous reconstruction lie within it.

Peer (39) made a histological study of 36 autogenous bone grafts from rib, tibia, and ilium in contact with unlike tissues (soft tissues). The bone grafts, whether cancellous or cortical, were replaced by fibrous tissue in 8 to 12 months after transplantation, the bone cells of the graft disappearing when the matrix was absorbed. This occurred re-



FIG. 66. Autogenous human rib bone with attached cartilage (chondrocostal junction) buried for 18 months. The rib bone without periosteum was fixed to the mandible and the cartilage was free in the region of the jaw joint. The bone, which became fixed to the host mandible, has become denser in structure but contains living bone cells on the basis of fixed and stained sections. The cartilage survived as would be expected. ×80.

gardless of the presence or absence of periosteum on the grafts or their thickness or thinness. Bone grafts transplanted in the vascular tissues of the neck and in muscle were absorbed just as readily as when they were transplanted into the relatively avascular subcutaneous fat of the abdominal wall. These findings do not agree with those of Mowlem. The author's interpretation is that rib, tibial and iliac bone grafts tend to lose their calcified matrix when they are transplanted into soft tissues regardless of the vascularity of the host bed. It is possible, of course, that bone grafts are retained in the nasal area although not in actual contact with bone, as reported by Carter and Mowlem, but absorbed elsewhere. If this is so, then the nasal tissues constitute a very special host site, which is favorable for the survival of rib and iliac bone grafts not in contact with bone.

Examination of early sections of rib, tibial and iliac grafts demonstrated that many of the bone cells in cancellous grafts (and sometimes in cortical grafts) survived transplantation as living cells, but that these cells apparently lost the ability to maintain their calcified intercellular substance, which was slowly invaded and replaced by host fibrous tissues over a period of about 12 months. The surviving bone cells simply disappeared among the invading fibroblasts after their matrix had been absorbed. One cannot say, therefore, that they died. They appeared as normal living osteocytes up until the last remnant of calcified matrix surrounding them had been removed.

On the basis of microscopic examination after complete removal and biopsy study of 16 autogenous bone grafts in contact with bone, Peer draws the following conclusions: Bone grafts from the rib, tibia, and ilium in contact with bone tend to retain their bony structure following transplantation, provided fixation occurs. The graft is joined to the adjacent host bone by osseous union. In cancellous bone grafts many of the cells in the graft may survive as living cells; just how many survive and how many are replacement cells from the host bone or its periosteum is impossible to say.

When the cells in rib and iliac bone grafts in contact with bone fail to survive transplantation, the graft structure tends to be absorbed and replaced by the process of creeping substitution from the host bone, its periosteum or possibly from the surrounding connective tissue. This replacement of bone is clearly demonstrated when boiled autogenous bone is transplanted in contact with bone. Rib and iliac bone grafts transplanted as simple onlay grafts to fill depressions and not subject to functional use formed osseous union with the host bone, but were greatly reduced in size over a period of about one year.

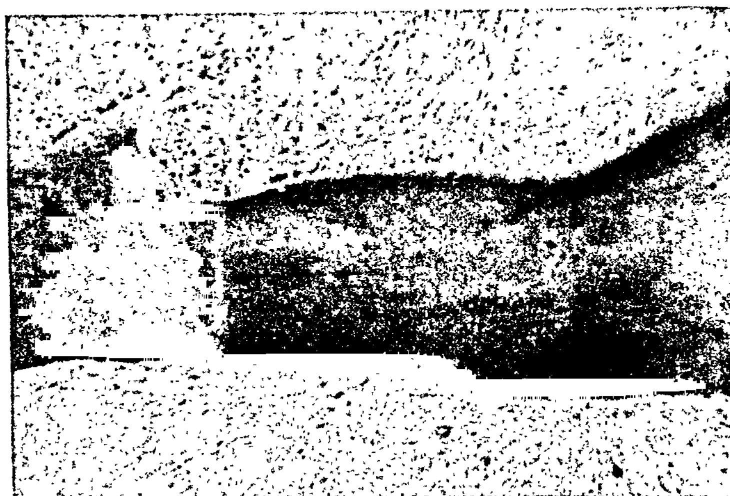


FIG. 67. Autogenous human iliac bone graft transplanted without periosteum to restore continuity for the mandible (in contact with bone). A segment of the graft was removed 2½ years after transfer. The graft at the time of transplantation was cancellous bone with cortex on one surface. Note that calcified matrix has become much denser in structure. Under higher magnification the bone cells appeared viable. Grafts in contact with bone and subject to stress and strain seem to retain their calcified matrix better than simple onlay grafts which serve to fill out contour. ×75.

Nasal, Septal and Turbinate Bone Grafts

In further study by Peer 31 human septal bone grafts, 4 nasal and 3 turbinate bone grafts, without periosteum, were transplanted in contact with unlike tissues such as abdominal and neck fat and muscle. *These grafts remained as bone.* All three types of grafts retained their normal bony structures with living cells until 5 years following transfer, as demonstrated by microscopic examination of stained sections.

The grafts become denser in structure following transplantation, but the parenchymal cells remain as living cells. Thus, septal, nasal and turbinate bone grafts (and possibly other membranous bone grafts) are able to maintain their calcified structure without contact with host bone.

Autogenous septal bone grafts killed by alcohol or heat are replaced by the host fibrous tissue in about 12 months, thus demonstrating the importance of the living osteocyte for survival of the graft structure. Peer also observed that the host fibrous tissue replacement is often associated with true bone formation in dead cartilage grafts, but that this does not occur in dead bone grafts in soft tissue sites.

In Elliott and Scott's case report (40) of compound fracture of the right frontal area of the skull, fragments were removed and immediately put in the deep-freeze unit in the blood bank, at −20 to −40°C. Primary healing of scalp wounds occurred, pneumocephalus developed, and after reoperation, a fistula was closed. Boiled fragments of preserved autogenous bone were replaced after ten weeks of refrigeration, resulting in primary healing. In a follow-up for 18 months, the postoperative course was satisfactory. The patient returned to full duty as a steward on an ocean-going vessel.

The bone and joint structures of a complete supernumerary toe and finger respectively were buried by the author[3] in the abdominal fat of the same infant. The toe had two bones and the finger, three bones, and all the joints were enclosed by the capsule coverings. The nails were retained on the terminal toe and finger bones and the periosteum was left attached to the bones. When examined radiographically and by palpation 22 months after transplantation, the bones and cartilage were found to have the same structure as at the time of transplantation. It is surprising to find that on palpation through the skin the bones can be readily flexed and extended, which indicates that the joint surfaces and capsules have survived and that the separate bones have not become joined together or absorbed and

[3] Unpublished data (Peer Clinic).



FIG. 66. Autogenous human rib bone with attached cartilage (chondrocostal junction) buried for 18 months. The rib bone without periosteum was fixed to the mandible and the cartilage was free in the region of the jaw joint. The bone, which became fixed to the host mandible, has become denser in structure but contains living bone cells on the basis of fixed and stained sections. The cartilage survived as would be expected. ×80.

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structure and they were greatly influenced in their interpretations by the published results on animal experimentation.

About the beginning of the present century Lexer and others used bone grafts clinically. They were impressed by the teaching of the great physiologist Ollier, and they emphasized his theory that bone grafts die but are regenerated from osteoblast cells in the periosteum and endosteum. Most surgeons at this time, therefore, subscribing to the osteoblastic theory of bone production were careful to transplant bone grafts with a periosteal covering on at least one surface.

Murphy in 1912 believed in the osteoblastic theory of bone production whether from the deep layer of the periosteum, or from the endosteum in the haversian canals and marrow cavity. He emphasized that the bone cells in autogenous grafts transplanted in soft tissue always die and that the graft structure becomes absorbed; autogenous bone transplants, with or without periosteum, in contact with living bone are united to the living fragments and act as a scaffolding for reproduction of new bone with living bone cells.

The majority of investigators, clinicians, and histologists at the present time believe at least partly in the osteoblastic theory of bone production described by Ollier, and few will disagree completely with the observations and conclusions by Murphy. *By 1912, therefore, it was known and generally accepted that autogenous bone grafts, with or without periosteum, retained their calcified structure when transplanted in contact with living bone.* The retention of the calcified structure was believed to be brought about through a convenient process of replacement from the living host bone. This process was called creeping substitution, by means of which the dead osteocytes in the bone graft were replaced with living cells and new calcified matrix formed to keep pace with the absorption of the calcified matrix of the graft. The new bone formation was believed to be affected by specific bone-forming cells, the osteoblasts, which migrated to the dead bone graft from the deep layer of the periosteum, from the haversian canals, and marrow cavity of the host bone.

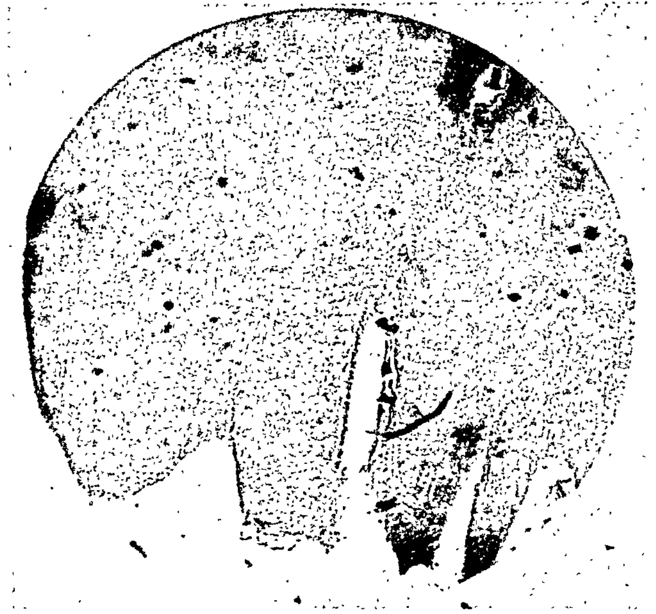


FIG. 69. Dense human tibial bone graft with periosteum buried in abdominal fat for 5 months. Note that bulk of calcified matrix is still present. The sparsely scattered osteocytes under higher magnification appeared as living cells in spite of the extensive decalcification process necessary in order to cut the graft. Another similar graft in the same patient could not be found 1 year after transplantation.

There was disagreement (and there still is) regarding the bone-forming ability of osteoblasts in the periosteum attached to bone grafts. *Most investigators believed that bone grafts could be successfully transplanted either with or without periosteum and this belief still holds today.*

Murphy[4] also observed that bone grafts transplanted in contact with soft tissues

[4] Murphy, it will be recalled, was a stormy petral among his Chicago medical confrères. He had great surgical ability, a tremendous energy and real talent as an investigator. He also had a very large surgical practice and a habit of stating his opinion regardless of how it affected others. He received honors abroad long before he was a member of his own county medical society.

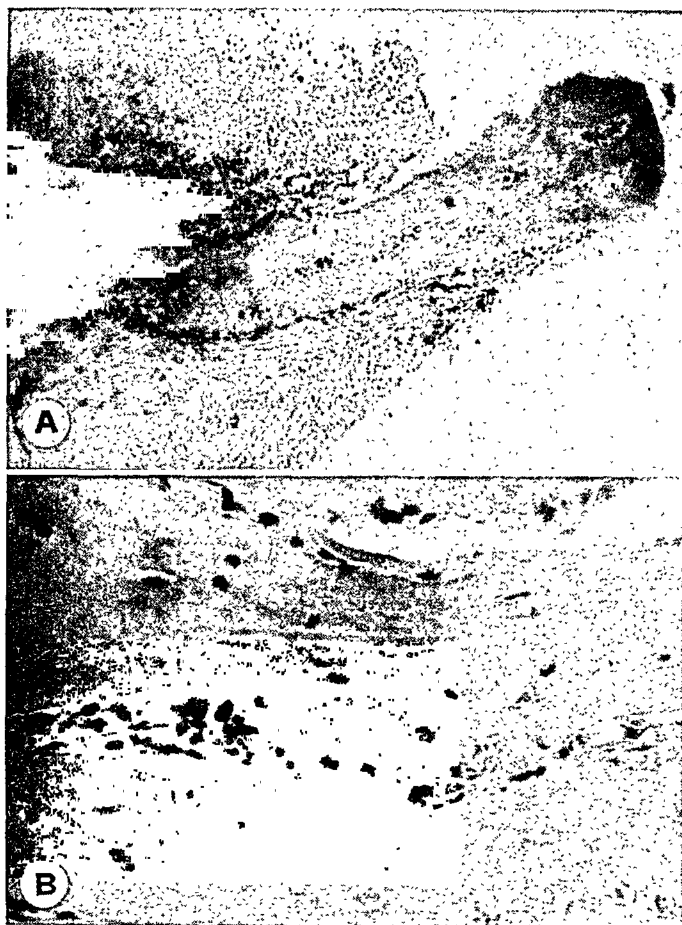


FIG. 68. A. Autogenous human rib bone buried in abdominal fat for 2 months. (Cancellous with cortex on one side.) ×80.
B. Higher power magnification of same graft buried in abdominal fat for 2 months. Note viable osteocytes which have apparently survived transplantation as living cells. ×340.

replaced by fibrous tissue. (This experimental work is previously referred to in Chapter 12, The Transplantation of Cartilage in Humans.)

RESUMÉ OF LITERATURE ON AUTOGENOUS BONE GRAFTS IN HUMANS

The factual data and interpretations in the literature on the behavior of autogenous bone grafts in humans at first appear hopelessly conflicting as did those on animal experimentation. Similarly, as one enumerates the voluminous findings, however, a rather definite and progressive order emerges from disorder.

About every ten years or so a surgeon or investigator with an intense interest in bone grafts appears on the scene. This individual assimilates the earlier findings of other men, makes numerous observations of his own, and eventually, in a paper or book, presents all of this material as a coordinated whole, which rather accurately states the acquired knowledge about the behavior of bone grafts at that time.

A study of the writings of such men, from Ollier to Albee, reveals that most of our present conceptions regarding the behavior of autogenous bone grafts were established by the year 1926. *The few newer contributions of importance since 1926 have been additions to this older work in certain aspects of bone graft behavior or of new bone formation not considered by earlier investigators.* The old concepts have not been proved false.

The earlier surgeons employed bone grafts clinically as occasional operative procedures in a few selected cases. They made clinical observations concerning survival of the graft

tive of whether they were creating it (Ollier) or destroying it (Leriche and Policard). A few giant-cell osteoclasts have also been observed. Apparently osteoblasts and occasional osteoclasts are present where bone is being formed. Whether they have an active rôle in the process or are merely onlookers is not definitely known at this time.

Albee[5] in his book (1915) and in later papers emphasized the importance of exact approximation and absolute immobilization in order to obtain bony union of the graft and host bone. Neuhof in 1923 agreed with Albee in believing that the "take" of a bone graft is enhanced by accurate approximation and immobilization of the graft, and present-day surgeons follow this practice.

Albee was convinced by wide clinical experience that the best transplant is a live piece of autogenous bone including the periosteum. Despite the current popularity of frozen and preserved homogenous bone grafts, there are few who do not agree with Albee, and most surgeons prefer autogenous graft to living or dead homogenous graft. Certainly the majority of clinicians and investigators believe that the presence of periosteum on a bone graft favors the early revascularization of the graft. Some men feel that the presence or absence of periosteum is not important but none believe that the presence of periosteum inhibits or prevents the take of a bone graft.

The earlier belief was that all or most of the osteocytes in a bone graft in contact with bone die, and that the graft structure is slowly absorbed. New bone cells and new calcified matrix are provided by osteoblasts which infiltrate the graft from the host bone. Mowlem reopened the question and concluded that a larger number of the osteocytes in cancellous bone grafts survive because of the open structure of the graft, which permits adequate and early nutrition for the cells. Contrariwise, most or all of the osteocytes in dense bone die because of nutritional difficulties following transplantation. Mowlem also emphasized the importance of a rich vascular bed for bone grafts as an important factor influencing survival of the graft even when it is not in contact with bone.

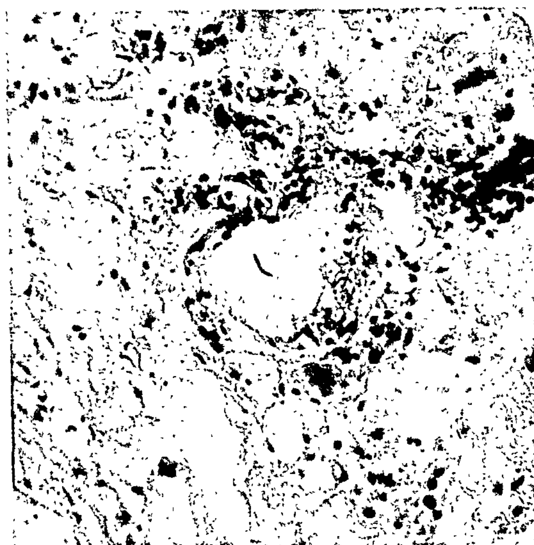


FIG. 71. Microscopic photograph shows the host fibrous tissue replacement of an autogenous rib bone graft buried for 10 months in neck fat.

All of the calcified matrix in the graft has been absorbed and the bone cells have disappeared.

Note giant cells, which may represent a coalescence or fusion of the evicted but living bone cells.

In a continuing series of experiments I have observed that different types of autogenous bone grafts in humans behave in different ways following transfer to various soft tissue sites. *Rib, tibial and iliac bone grafts, whether cancellous or cortical, are absorbed after transplantation in abdominal and neck fat and in muscle regardless of the presence or absence of periosteum. On the other hand, grafts from the nasal bones, nasal*

[5] Albee was very much like J. B. Murphy of Chicago. He was a fine surgeon with a large practice, an accurate investigator and a good teacher. The author knew him personally and admired his many good qualities and great talents. He did have a habit of "making the headlines" at a time when public relations for surgeons were not accepted as they are today. He was therefore not popular with his confrères. Albee was a master surgeon and the best authority on bone grafts during his time.



FIG. 70. Section represents a graft of autogenous cartilage and rib bone taken at the chondrocostal junction, and buried in chest fat. Removed and examined 6 months following transplantation.

An equal amount of cartilage and bone were originally contained in the graft. The cartilage has survived, retaining its same general size but the bone on the right has been reduced to a very small spicule.

High-power magnification in other sections demonstrated that the host fibroblasts were in the process of completely replacing the remaining calcified structure of the bone.

were absorbed and replaced by fibrous tissue rather than by bone. At the present time it is known that this is so concerning the types of bone grafts that Murphy transplanted. *He did not transfer septal, nasal or turbinate bone grafts, all of which tend to retain their calcified structure in soft tissue beds.*

Carter, who was an otolaryngologist and interested in the repair of saddle-nose deformity, had made an important observation in 1911. He noted that rib bone grafts, with or without periosteum, retained their bony structure after transfer to the nose, although the graft was not actually in contact with the nasal bones. Since it was known that rib-bone grafts transplanted in soft tissues elsewhere are absorbed and replaced by fibrous tissue, it appears that the nasal tissues constitute a transplantation site especially favorable for the survival of rib-bone grafts. Mowlem in 1941 noted that iliac bone grafts also retained their calcified matrix following transfer to the nose although the grafts were not in contact with bone. Thus, Mowlem's recent observations confirm Carter's findings reported in 1911, which attracted little attention at the time.

Leriche and Policard in a series of articles, later consolidated in delightful book form (41), advanced their mesenchymal theory of bone formation in opposition to Ollier's osteoblastic theory. They believed that the osteoblasts were not bone-forming cells but, instead, osteogenesis occurred through the activity of undifferentiated mesenchymal cells. A bone transplant succeeds only because it dies and provides the necessary source of calcium from which the host connective tissue is capable of recreating bone.

Present-day opinion tends to support Ollier's osteoblastic theory rather than the mesenchymal theory of Leriche and Policard. Some believe that the osteoblasts are important during the normal growth of bone but that mesenchymal tissue is the main agency for osteogenesis after growth is complete.

It appears to the author that too great a distinction is drawn regarding the specificity of osteoblasts and their fixed locations in the deep layer of the periosteum (cambium layer), the endosteum of the marrow cavity, and the haversian canals. There is considerable evidence that the mesenchymal cell from which the osteoblast or bone-forming cell is probably derived is more widely distributed and possibly exists in almost all of the body tissues. One example is the fact, noted by the author many times, that new bone can occur in preserved homogenous cartilage grafts and in fresh autogenous grafts buried in fat and muscle. Certainly there was no periosteum nor haversian canals nor marrow cavities in the vicinity of these grafts, but still new bone formation took place. Cells appearing exactly like osteoblasts were present next to the bone irrespec-

It is difficult to explain why autogenous septal, nasal and turbinate bone grafts retain their calcified matrix following transplantation in soft tissue sites, whereas autogenous rib, tibial and iliac bone grafted in similar sites are absorbed and replaced by fibrous tissue. One notes however, that the septal, nasal and turbinate bones all lack regenerative powers, that is, they lack the ability to replace portions of bone that have been removed. In this way, septal bone does not reform after a submucous resection operation. The nasal bones, fortunately, do not form a new bony hump after a rhinoplasty, and the turbinate bone does not reform after a turbinectomy. Rib, tibial and iliac bone all have some regenerative power. Perhaps the cells in bone grafts that lack regenerative powers are endowed with a tenacious ability to retain their calcified matrix regardless of contact with bone, whereas the cells in bone grafts with regenerative powers do not appear to have this ability. This hypothesis would suggest that the bone cells at some stage in their differentiation have acquired different abilities which determine their later action under the conditions of free transplantation.

An impressive fact regarding the survival of fresh autogenous septal, nasal and turbinate bone grafts in humans is the consistency with which it occurs. Thirty-eight of these grafts without periosteum were transplanted to soft tissue sites, *and all 38 retained their calcified structure.* All of these grafts were removed at intervals from 3 days to 5 years after transfer, and all appeared like normal living bone containing *living osteocytes.* Osteoblasts and osteoclasts were not present as recognizable cells in the stained sections, and there was no evidence of new bone formation or of bone absorption.

Autogenous rib, tibial and iliac bone grafts in soft tissue sites were removed at intervals of 2, 4, 6, 8 and 12 months after transfer. I was extremely interested to note the ab-



FIG. 73. Autogenous human septal bone graft buried for 3 weeks. The blood vessels in septal bone run through channels in the calcified matrix. After transplantation it is probable that only the established vascular channels in the bone are used. Circulation is established through end-to-end anastomosis between host and surviving graft blood vessels, as in all other free grafts. Note normal appearance of osteocytes in the graft.

sence of osteoblasts and osteoclasts in the numerous areas on the graft surface where absorption was taking place. These areas of absorption were occupied almost entirely by young active-looking fibroblasts accompanied by numerous blood vessels. The fibroblasts appeared to be the cellular agency active in the removal of the calcified matrix; the picture was much like that seen in the host tissue surrounding cartilage grafts in the process of absorption.

The bone cells in the disintegrating grafts *appeared like living bone cells as long as they were surrounded by their bony matrix.* In areas where the matrix had been removed they could not be identified in the dense multitude of fibroblasts.

The majority of these rib, tibial and iliac bone grafts consisted of medullary bone with cortex on one side. Since the osteocytes can and do survive in these grafts when they are transplanted in soft tissue, it seems reasonable to assume that they may also survive when the grafts are transplanted in

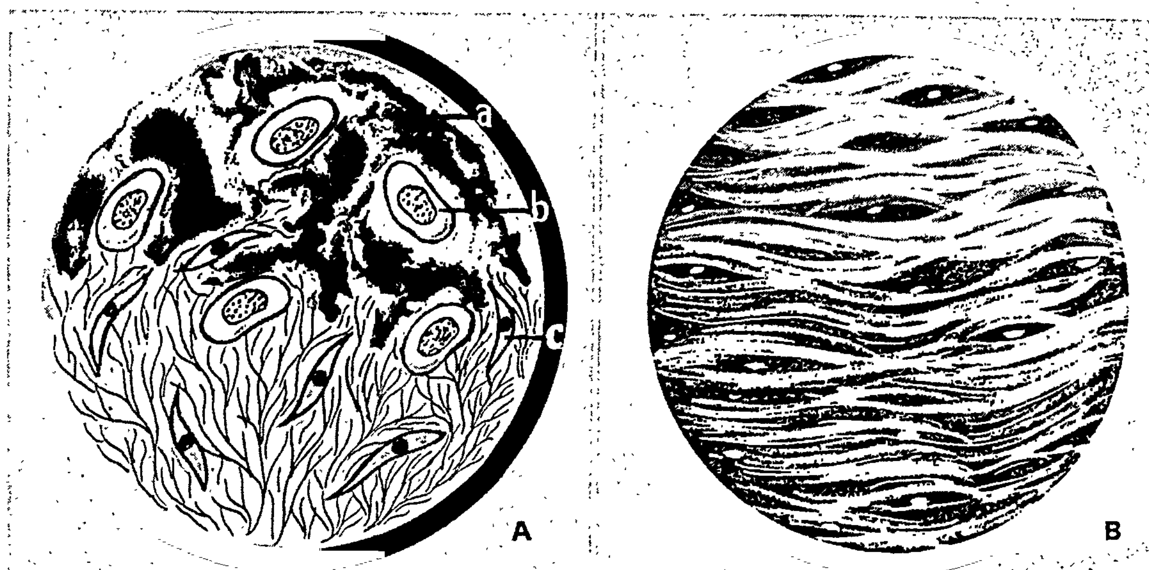


FIG. 72. A. Drawing demonstrates the typical appearance of an autogenous rib, tibial, or iliac bone graft transplanted in contact with unlike tissue and buried for 4 to 6 months. The host fibroblasts are in process of replacing the calcified matrix of the bone graft. The bone cells have survived transplantation as living cells.

B. After about 8 months the host fibroblasts completely replace the calcified matrix of the bone The bone cells have disappeared among the fibroblasts which have replaced the calcified matrix of the bone. One cannot say that the bone cells have died. They survive the initial shock of transplantation as living cells and may still be alive. (Drawing by Miss Ruth Pullen, R.N.)

septum (vomer and ethmoid) and the turbinates, transplanted without periosteum, retain their calcified structure after transfer to similar transplantation sites.

Microscopic examination of septal bone grafts removed 5 years after transfer demonstrated that the osteocytes survived as living cells. The matrix appeared to have become more compact and had a denser structure. The grafts were surrounded by a thin connective-tissue capsule but no osteoblasts nor osteoclasts were seen.

Grafts removed at 4 to 8 days after transfer showed evidence of a circulating blood supply, indicating that early end-to-end anastomosis between host and graft blood vessels had occurred. Examination of grafts buried for 3, 7, 14, and 21 days *indicated that the endothelial cells lining the blood vessels in the grafts survived as living cells. Thus, it may be stated that the vascular system in the grafts survived transplantation and continued to function.* There was no evidence of penetrating host capillary growth into the grafts. *The vascular system seems to survive in most successfully-transplanted free grafts.* In soft tissue grafts, such as fat, dermal and surface skin grafts, a penetrating host capillary ingrowth does occur, but the earliest circulation is established by anastomosis between the severed ends of host and graft blood vessels. There is good evidence that this remains as the permanent circulation for the graft.

Autogenous rib, iliac and septal bone grafts killed by heat and by preservatives before transfer to soft tissues were absorbed and replaced by fibrous tissue in about 12 months. This is about the same period of time required for the absorption of fresh rib and iliac bone grafts in soft tissue sites. Fresh septal bone grafts with living cells in similar locations retain their calcified matrix.

The Graft

Role of Periosteum

The presence of periosteum on a bone graft is not essential for successful transplantation. Most investigators agree that grafts with periosteum obtain an earlier blood supply than those without periosteum, but that the calcified structure of the graft remains regardless of the presence or absence of periosteum if the transplantation site is favorable for the particular graft.

Fate of Osteocytes

The cells in septal, nasal and turbinate bone grafts tend to survive and retain their calcified matrix after the grafts are transplanted in soft tissue sites.

The cells in rib, tibial and iliac bone transplanted in abdominal and neck fat and in muscle also survive until their calcified matrix has been absorbed, which requires a period of 8 to 12 months after transfer. The bone cells then disappear in the multitude of invading fibroblasts and cannot be identified. *One cannot accurately state that they are destroyed.*

Probably many of the osteocytes in cancellous rib, tibial and iliac bone transplanted in contact with bone also survive and maintain the calcified structure of the graft. Osseous union occurs between graft and host bone and small portions of the graft may be replaced by creeping substitution. When the osteocytes in all or part of the grafts fail to survive, this dead bone is absorbed and replaced by creeping substitution from the host bone, host periosteum, or host connective tissue.

Living osteocytes are essential for the survival of septal, nasal and turbinate bone grafts in soft tissue sites. I have noted that when the osteocytes in these autografts are killed by heat or preservatives before transplantation the calcified matrix is absorbed in 8 to 12 months.

Some authorities believe that the bone cells all die and that the graft structure is completely repopulated by new bone cells from the host bone, from its periosteum, or from mesenchymal cells in the surrounding host connective tissue. Others agree that many of the graft osteocytes in cancellous bone survive as living cells which continue to service and maintain their calcified matrix.

It appears from substantial evidence that osteocytes in autogenous bone grafts in favorable transplantation sites survive as living entities. This is specially true of cancellous rib and iliac bone grafts in contact with bone, which have an open structure, and also of cancellous grafts with a thin cortex covering on one surface. Many of the cells in thick cortical grafts from the tibia may fail to survive because of inadequate nutrition after transfer. These may be replaced by new osteocytes from the host bone. Such grafts ultimately will consist of a composite structure containing new elements from the host bone and surviving elements from the graft.

Factors in Survival of Bone Grafts

Early Nutrition and Vascularization in Bone Grafts

Tissue fluid exuding from the host site serves to prevent desiccation of the graft cells during the first few days, but this may not be as important a factor in providing nourishment for remotely located cells as has been supposed. There is evidence that the cells in bone grafts and in other tissue grafts can remain viable in the absence of any circulating fluid up to periods of four days or more following transplantation if they are kept moist.

The blood vessels in free bone grafts survive transplantation and *circulation is established through end-to-end anastomosis between host and graft blood vessels.* One does



FIG. 74. Autogenous human septal bone and septal cartilage grafts buried in abdominal fat side by side for 3 years. The author expected at this time (some years ago) that the bone would be absorbed but that the cartilage would remain. As shown in the microphotograph both the septal bone graft and the septal cartilage retained their same general bulk, and the cells in each appeared viable in the fixed and stained sections. This experience induced the author to transplant other facial bones in soft tissue sites. ×75.

contact with bone. *The process of creeping substitution of the graft structure from host bone may not be as active in autogenous bone grafts as has been supposed.*

I have also noted that simple onlay grafts in contact with bone tend to diminish in size over a period of time although they obtain bony union with host bone. Bone grafts subjected to stress and strain tend to retain their same general size. *Thus, functional use appears to influence the retention of calcified matrix in a graft.* Grafts (rib and iliac) successfully transplanted to fill a skull defect often lose much of their original size over a period of time, so that the depression again becomes apparent although these grafts appear to have established bony union with the sides of the skull bone. The idea that function affects bone is an old one, which has been noted by a number of investigators and is sometimes stated as Roux's law.

Cancellous rib and iliac bone grafts transplanted in contact with rib bone as simple onlay grafts resulted in osseous union with the host rib bone by roentgenographic evidence. When the patient's chest was reopened one year later the grafts were still present but were greatly reduced in size.

FINAL SUMMARY OF BEHAVIOR OF AUTOGENOUS BONE GRAFTS IN HUMANS

Though involving repetition, recapitulation may well serve to clarify the behavior of oseous transplants. Based on the available knowledge at this time the fate of autogenous bone grafts in humans is as follows.

Transplantation Site

Many types of bone grafts, such as rib, tibial and iliac grafts, must be transplanted in contact with bone in order to survive. When transplanted in abdominal and neck fat or in muscle, the calcified matrix of these grafts is slowly invaded and replaced by fibrous tissue. An exception to this statement is the reported fact that rib and iliac bone grafts can survive in the nasal tissues when not in actual bony contact with the nasal bones. Contrariwise, septal, nasal, and turbinate bones tend to retain their calcified structure after transfer to soft tissue sites such as abdominal and neck fat and muscle. It is probable that some other facial and skull bones have this same capacity to retain their calcified matrix after transfer to soft tissue sites. One hyoid bone graft in muscle can be palpated four years after transplantation.[6]

[6] This graft was buried by Dr. Edgar Cardwell of Newark.

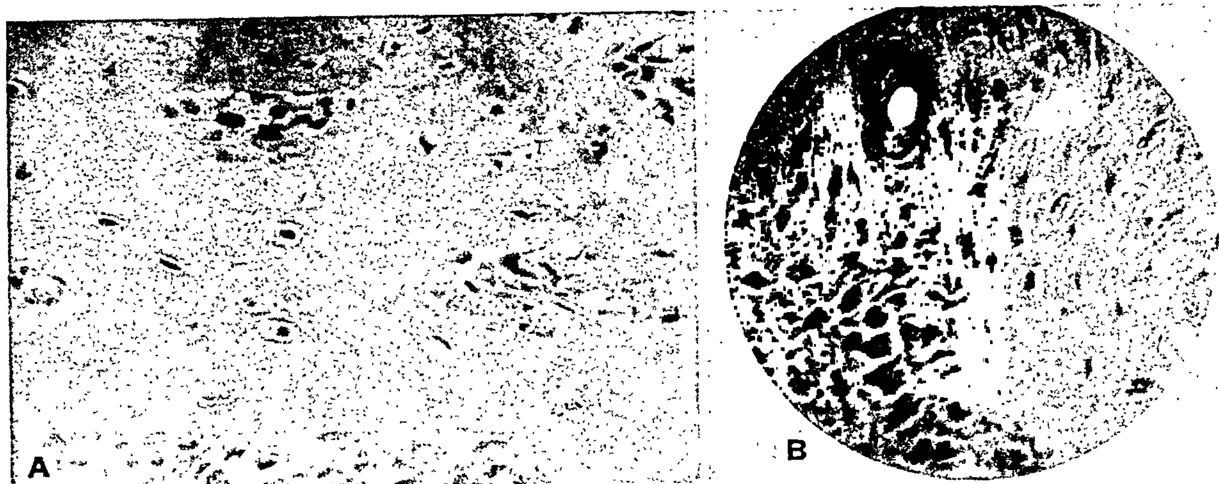


FIG. 76. A. Autogenous human nasal bone graft buried in abdominal fat for 5 years. Note very normal apperance of the bone cells. ×325.
B. Human septal bone graft buried in abdominal fat for 8 months. Note normal apperance of osteocytes.

are the types most commonly used in bone grafting, require immobilization as well as contact with living bone if they are to survive.[7] Apparently, absence of movement at the point of contact between graft and host bone is essential for bony union, and this bony union is necessary for the retention of the calcified structure. Immobilization is not necessary for the survival and retention of the calcified structure in septal, nasal and turbinate bone grafts in soft tissue sites. These bone grafts behave much like cartilage grafts.

Functional Use

There is abundant evidence that autogenous rib, tibial and iliac bone grafts tend to retain their calcified matrix better when they are subject to forces of active function such as stress and strain. In this way bone grafts establishing continuity in a long bone or in the jaw often retain all or most of their original bulk. When these same bone grafts are transplanted as simple onlay grafts and not subject to stress and strain, a considerable part of the calcified matrix is frequently lost over a period of time. Thus, onlay grafts on the outer surface of the jaw and grafts utilized to replace absent portions of the skull bone may be reduced in size even though they establish bony union with the host bone. On the other hand, autogenous septal, nasal and turbinate bone grafts retain their calcified matrix after transfer to soft tissues, where they have no function whatever.

[7] A possible exception is a transplantation site such as the subcutaneous tissue of the nose, where the rib and iliac bone grafts may survive when not in actual contact with bone.

Growth of Bone Grafts

Interstitial growth of bone is a physiological impossibility because the bone cells are encased in a rigid calcified matrix which is not malleable. If the osteocytes had the capacity to deposit calcium (and this has never been demonstrated) there would not be any space for this deposition since the surrounding calcium matrix is not capable of expansion.

Growth in long bones takes place at the ends through replacement of the epiphyseal cartilages and on the outside surface by appositional deposit through the activity of osteoblasts, mesenchymal cells, or some unknown agency. In bones such as those of the skull and palate with no epiphyseal car-

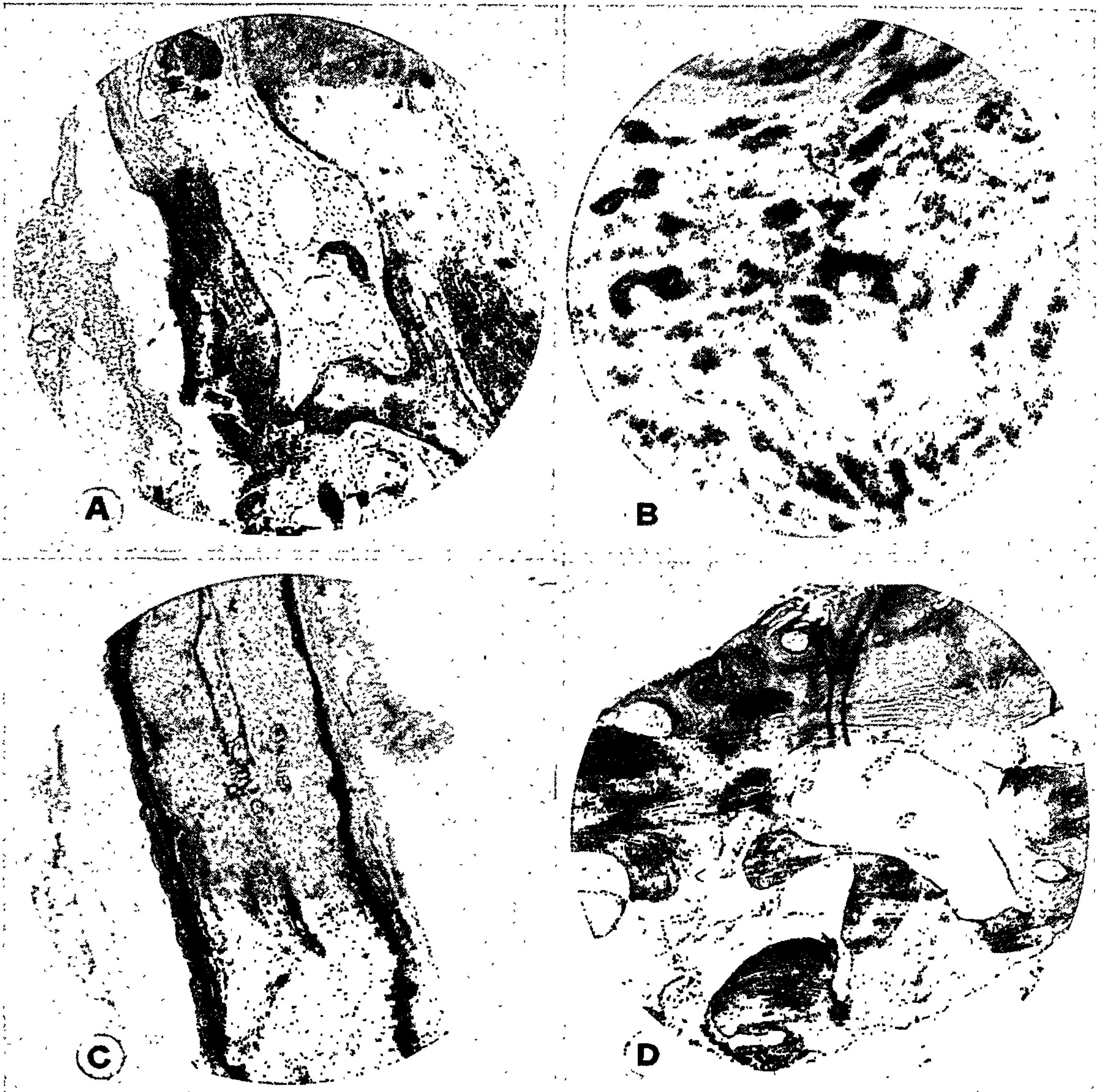


Fig. 75. A. Septal bone graft in fat 3 months after burial. Bone appears normal.
B. High power view of septal bone graft buried 3 months. Note living bone cells.
C. Septal bone graft in muscle buried 8 months. Bone structure intact.
D. Septal bone graft in fat buried 4½ years. Normal bone structure which has become very dense but contains occasional living bone cells.

not observe a penetrating ingrowth of host capillaries into the hard outer substance of septal bone grafts, hence circulation takes place through the established opening in the bone. In cancellous bone grafts, penetrating capillary ingrowths do occur together with anastomosis between host and graft blood vessels. I have noted that endothelial cells lining the blood vessels in bone grafts show little degenerative change following transplantation and circulating blood has been observed in the vessels of septal bone grafts buried for only three days. The larger blood vessels become occluded by clotted blood but the endothelial cells remain viable. The blood clot is often organized and absorbed and the blood vessel becomes recanalized and again serves as a channel for circulating blood.

Importance of Immobilization

Rib, tibial and iliac bone grafts, which

sues throughout the body (Leriche and Policard)?

The only safe statement to make about the mechanism of osteogenesis is that the exact *modus operandi is not known at this time.*

On the positive side one may say that the presence of living cells and blood vessels is necessary for bone formation, since it never occurs in the absence of living cells and a rich blood supply. In any event, *bone formation is a vital phenomenon* irrespective of the chemistry involved in the process. The deposition of calcium salts may be a chemical reaction, necessitating certain mineral and enzyme ingredients, but it does not occur without a chemist in the guise of a living tissue cell. This cell is "the ghost behind the machine." If one likes to speculate, it appears in pursuing scientific research that a point always arises ultimately when the investigator must confess that he can no longer understand and therefore is unable to explain. Walsh (43) in his very complete discussion concerning osteogenesis, states that the physicochemical theory of bone formation—which is based on the ability of an excess of calcium salts, in the presence of an adequate blood supply, to stimulate the formation of bone from undifferentiated mesenchymal tissues—is *not capable of being substantiated at the present time.*

The *inability to obtain bone* from implantation of salts in muscles, the fact that calcification occurs from the minerals of the blood, the inability of the weak acid of the hematoam to dissolve the minerals in the bone fragment, all point to the conclusion that bone formation and calcification *is a vital process* and one that is peculiar to vertebrates. It does not occur in some animals of lower phyla, even though they possess the ability of calcifying various structures. The nature of the stimulating force, which is not calcium nor phosphatase, is unknown at this time.

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tilages, growth in length is accompanied by osteogenesis at the suture lines.

Growth of bone grafts in contact with bone, therefore, can take place only by the *deposition of bone on the exposed surfaces of the graft which are in contact with soft tissues.* Most authorities agree that bone grafts in growing children tend to increase in size through their appositional growth.

Authorities have disagreed regarding the particular cell responsible for bone growth and bone regeneration in bone grafts. At the present time most of our histologists accept the osteoblastic theory, which holds that bone formation can take place only through activity of the osteoblast cell located in the deep layer of the periosteum and in the endosteum of the bone marrow and haversian canals. The main opposing theory is the mesenchymal theory of Leriche and Policard, namely, that bone formation occurs through the agency of undifferentiated connective-tissue cells which are widely distributed throughout the body. The exact mechanism for the formation of the collagenous matrix substance in bone and the mode of deposition of salts in this matrix are not known. The mechanism by means of which bone is absorbed is likewise unknown. It is generally agreed that the osteoclast giant cell is not the cellular agency responsible; the author has often observed bone absorption in bone grafts with a complete absence of osteoclasts.

Septal, nasal and turbinate bone grafts after transfer to soft tissue sites behave like cartilage grafts in that they retain their same general size. They do not appear to increase in bulk or grow when young grafts are transplanted in growing children, which is also true of young human cartilage autografts.

Healing of Bone Grafts in Contact with Bone

The various stages in the healing of bone grafts in contact with bone are similar to those in the healing of fractures. A hematoma forms, and after 24 to 48 hours the blood in this hematoma coagulates and fibrin appears. The fibrin is invaded from the periosteum, endosteum, and haversian canals of the host bone by cells which resemble rather plump and young fibroblasts. Similar-appearing cells from the host connective tissue may also invade the fibrin, as suggested by Leriche and Policard in their mesenchymal theory. At any rate these young fibroblasts, as they appear to be, are accompanied by numerous blood vessels; as the fibroblasts or osteoblasts mature, collagenous fibers are laid down around the new blood vessels. Almost immediately minerals are deposited in the inorganic collagenous matrix and the fibroblast cells in the matrix become trapped as bone cells or osteocytes. According to Urist and McLean (42), the repair of bone always occurs by preliminary formation of *fibrous or fibrocartilaginous model of the fracture site.* When a cartilaginous model is formed it is later replaced by new bone through the activity of cells in the bone, periosteum, or surrounding connective tissue.

During the process of bone formation the hematoma, which is at first acid in reaction (pH 4.7), later becomes alkaline, and the levels of calcium and phosphatase concentration increase greatly.

There is a difference in opinion concerning the cause and exact nature of osteogenesis. Among other controversial issues investigators are not in accord as to whether the cell plays an active part in osteogenesis through the activity of enzymes or whether the deposition of calcium salts is a simple physicochemical reaction.

The authorities who believe that the cell plays an active part in osteogenesis disagree regarding the identity of this cell. Is it the osteoblast located in the cambium layer of the periosteum, the haversian canal, and endosteum (Ollier)? Or is it an undifferentiated mesenchymal cell present in most tis-

CHAPTER 18

Homogenous Bone Transplants in Humans

The first successful transplantation of bone from one person to another was reported by Macewen (1) of Glasgow in 1878. Since that time there has been extensive transplantation of homogenous bone in humans, and reports of excellent clinical successes with bone homografts in various forms have appeared in the literature.

REVIEW OF LITERATURE ON FRESH HOMOGENOUS BONE TRANSPLANTS

Poncet (2) in 1887 reported clinical trial and transplantation of the first phalanx from the large toe of an amputated leg to an ununited fracture of the right tibia in another patient. Only union by fibrous tissue was evident, with beginning consolidation.

Barth in 1895 and 1896 claimed that all of a bone transplant died and therefore homogenous and heterogenous transplants would be as successful as autogenous ones (3). Later he retracted this assertion (4).

After removal of tuberculous granulation from the cranial vault in a child, Büdinger (5) (1900) applied two plates from the spongiose calcaneus of an amputated femur to the bony defect. The wound healed *per primum*, and no essential change was observed in the implanted osseous pieces during subsequent years—the spongiose bone being replaced by compact bone which was not distinguishable from its surroundings. There was no recurrence of the tuberculous lesion. The calcaneus bone adapted itself so that new bone formation occurred.

Joint transplantation was first clinically applied by Lexer in 1907. After resection of the upper third of the tibia with its articular surface for sarcoma, he transplanted a similar portion of tibia which had been removed from a freshly amputated limb (6).

From clinical observations on humans as well as from experiments on 146 animals, Axhausen (7) in 1909 and 1911 concluded that periosteal-covered autogenous grafts were best. The succeeding order of efficiency he listed as follows: 1) fresh osteoperiosteal homogenous grafts, 2) fresh autogenous grafts without periosteum, and 3) fresh homogenous grafts without periosteum.

The most prominent case of success with fresh cadaver bone transplant is Kuettner's, reported in 1911 and 1913. He implanted a whole upper femur removed from a cadaver 35 hours after death in the defect left by resection of the upper third of the femur for chondrosarcoma. When the patient died from metastases 13 months after the operation, the specimen showed good union, a newly-formed periosteum, and the reattachment of the musculature to the transplant.

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Smith's belief that homogenous bone grafts could be advantageously used in patients in whom a satisfactory autogenous graft could not be obtained.

Boyd (15) (1939) reported on 97 operated patients with congenital pseudarthrosis of the tibia, with 31 per cent successful results. He found the blood group was of no significance. Boyd's successful transplantations were obtained by the use of double coapting homogenous bone grafts. In the cases in which a single graft was fixed with a wire loop all were failures. Thus it appeared that the operative procedure was of more importance than the type of bone used.

Ghormley's experience (16) (1942) with fresh homoplastic bone transplantations was limited to 19 operations in 14 patients, 10 of which were successful. He believes that bone grafts must become attached to the host by granulation tissue; and granulation must invade the graft. This invasion in cortical grafts will be delayed because of relative impermeability of the graft. During this period the graft may undergo apparent death. After vascularization the graft may appear to atrophy owing to increased circulation. Ultimately both cortical and cancellous grafts become restored to normal consistency.

Ghormley and his colleagues used cortical grafts probably more widely than any other type. According to their experience at the Mayo Clinic the donor does not necessarily have to be a close relative nor does the blood have to be matched. In one case a piece of bone from a donor not related was preserved in alcohol for a time before being used successfully as a graft. Ghormley believes that homoplastic transplantation does have a place in the occasional case in which bone is desperately needed and for some reason or other the patient is in no condition to withstand autotransplantation.

In Henry's (1948) patients all fresh homografts were taken from male relatives of the patient and were syngenesioplastic grafts. Successful results from the use of homografts led him to feel that the method warranted wider application in certain conditions (17).

Harbin and Liber (18) (1949) reported cases illustrating the value of each type of graft—the full thickness inlay, the cortical onlay, the osteoperiosteal, the medullary, the sliding and the massive transplant grafts. It is their opinion that if growth and revascularization are judged to be any indication of life, then a large percentage of fresh homotransplants continue to live as such.

PRESERVED HOMOGENOUS BONE TRANSPLANTATION

Transplants of boiled or dead homogenous bone grafts had been applied previous to the use of freeze-preserved tissues in surgery. Barth (19) reported that he had had nothing but failures from the use of boiled bone. Lexer thought that boiled bone, obtained from a cadaver, or fresh bone which had been sterilized, acts as does a foreign body, slowly undergoing substitution (20). Kausch (21) (1906, 1910) obtained a bone transplant from a leg amputated because of fresh complicated fracture. The periosteum was stripped from the donor bone and the medullary cavity was curetted; the graft was then decalcified by extracting with alcohol and ether, and by boiling. The grafted bone was implanted and fixed with ivory pegs in the tibial head where a myelogenous sarcoma had been resected, with preservation of the fibula. Healing occurred by primary intention. Kausch stated that to his knowledge this is the largest transplanted dead bone that healed in firmly, namely, 9 cm. long. At the time of resection for recurrence of the osteosarcoma 9 months later, the specimen showed perfect union between the recipient bone and the donor bone.

Alexis Carrel (22), at the beginning of the twentieth century, laid the foundation for the application of preserved tissues in sur-

Staining showed that the bone cells had died and replacement was extensive (8).

Borst (9) in 1913 ascribed the failure of homogenous and heterogenous transplantation to the extreme specialization of the cells in higher animals. With advance in development and specialization the cells become more and more interdependent. Borst constructed a theory of "biochemic" differences to account for the opposing forces to homotransplantation and heterotransplantation. In many cases the transplanted tissue serves merely as a conductor or model for the regenerated bone.

Lexer's noted case was that of a 20-year-old girl whose knee joint was resected and replaced by a homograft from a freshly-amputated limb. Permanent healing was evident clinically 6 years after operation. Roentgenographically, changes due to absorption and excessive growth were seen at the site of union between the graft and host tissue (10).

In a case reported by Wade (11) (1920) the patient had a myeloid sarcoma of the upper end of the shaft of the humerus, which had caused a fracture; the head and upper portion of the shaft were removed; a cuff of periosteum detached from the shaft being left attached to the lower portion. A segment of homograft from an amputated limb was implanted, a portion of the articular cartilage of the femur on the graft was laid in contact with the glenoid cavity, and the lower end of the graft fitted into the periosteal cuff attached to the upper end of the unremoved portion of the shaft of the humerus. The functional result was excellent.

Neuhof (12) (1923) referred to reports of excellent results from the use of bone homografts taken from stillborn babies. As he pointed out, despite general agreement regarding the histologic picture that develops in the homograft and the host tissues, the interpretation of just how this occurs has given rise to controversial comments. He interpreted it as follows: A layer of new connective tissue is laid down following a fibrinous exudate. Callus derived from the host tissue units the graft and the host bone. In the neighborhood of the transplant the muscle or other tissues rapidly adhere to the surface of the graft. When the transplant retains its periosteum, union with the surrounding connective tissue is rapid and the layers of the periosteum are freely supplied with blood. After 6 days, however, no difference can be noted between vascularization of transplants with or without periosteum. The cells of the periosteum show degenerative processes for the most part. Approximately 3 days after transplantation the nuclei of the bone cells in the central part of the graft begin to disintegrate, and there is evidence of absorption of the dead bone. The connective tissue around the blood vessels becomes calcified and osteoblasts appear. The blood vessels and bone marrow of the transplant also show degeneration (12). As viewed by Neuhof, under favorable circumstances the graft structure with its dead bone cells is absorbed and replaced by the process of creeping substitution, so that ultimately the bone at the graft site is the patient's own bone.

After resection of a radius for sarcoma the radius from an amputated arm was transplanted by Ellmer and Schmincke (13) (1925) into the defect. The homogenous transplant proved to be a perfect substitute. The clinical results could not be differentiated from those in an autogenous transplant but after a fracture in 1924 consolidation was defective and pseudarthrosis resulted.

Homogenous bone grafts were used successfully by DeForest Smith (14) in 1937 on several patients with osteogenesis imperfecta. These grafts had had the periosteum removed and consisted of cortical and medullary bone. Ununited fractures in the lower extremities were corrected with onlay grafts from the tibia of donors. It was DeForest

bone or homogenous human bone is freed of fat, connective tissue, and protein but not freed entirely of all collagenous matrix.[1] Another grafting material devised by Orell is "os novum," which is produced by implanting a long narrow os purum splint of suitable shape subperiosteally over the anteromedial surface of the tibia.[2] The os purum splint is absorbed and replaced by new bone, and this new bone—the os novum—is excised and transplanted to the desired place. Orell used homogenous bone treated as described in 50 patients to fill defects, to lessen risk of deformities and to fix skeletal parts to one another. Immature living bone tissue (with great proliferative power) was employed by him in cases in which extraskeletal connective tissue separates two bones which are to be joined through transplantation.

Inclan (29) of Havana in 1942 reported on the use of autogenous and homogenous preserved bone as grafting material. In 74.4 per cent of 52 patients the results were excellent or good. The bone grafts were preserved in citrated blood, saline, and in saline and blood in an ordinary refrigerator.

Hellstadius (30) (1942) concluded from his experiments that os novum would survive in pedicled grafts and was rapidly osteogenic.

As reported by Goff (31) (1944), in a study of 80 operative procedures on 61 patients in whom os purum implants were used, the rate of fusion appeared to be slightly slower and the amount of bone fused somewhat less in size and thickness than in controls with autografts. These operations were for spine fusion, peg fixation in cancellous bone, intramedullary grafts in connective osteotomies, and shelf operation for congenital dislocation of the hip. Goff ascertained that an excess amount of os purum appears to be a long time in becoming absorbed and may produce slight serous discharge for several months. Furthermore, os purum implants, unless under physiological stress and strain and surrounded by osteogenic tissues, will be resorbed and disappear. He believes, however, that os purum has a definite place in bone surgery. Walsh also believes that os purum has a clinical place (32).

In 1947 Wilson (33) reported that there was no evidence of cells surviving in either autogenous or refrigerated homogenous bone transplants. The preservation of fresh autogenous bone material by refrigeration for later use in operations is safe and practical. Wilson experimented with homogenous bone from all sources, particularly from the iliac crest, which had been transferred to the refrigerator. In all cases the preserved bone seemed to behave identically like fresh autogenous bone. There was no evidence that the cells in transplanted bone survived in either case. In both autogenous and preserved bone transplants the bone is resorbed and transformed into living bone entirely as the result of the action of the host tissues.

Bush (34) in 1947 obtained excellent results from frozen homogenous bone transplants used in 67 operations with the exception of 4, as demonstrated by direct and roentgenographic examination. The blood type appeared to have no influence on the successful use of homografts of bone, nor did the Rh factor influence transplantation results. Bush held that in deep freeze the grafts may be preserved indefinitely. Biopsies were removed from fused areas in which homogenous bone had been employed. Microscopic studies showed bone spicules to be dead but surrounded by connective-tissue cells, with osteogenesis taking place by metaplasia of these cells. Homogenous bone, Bush goes on to say, seems to form a trellis and to act as a stimulus for new-bone formation, and pro-

[1] The ends are sawed off, and the bone is soaked in salt solution, the connective tissue removed by soaking in warm potassium hydroxide, the fat is extracted and the whole cleansed.

[2] When the material is excised from one to several months later, profuse growth of new soft vascular bone is found in the clefts between the periosteum and the tibia.

gery. He cited Tuffier (1910–1911) as preserving in an ordinary refrigerator pieces of human fat, bone, cartilage, and periosteum which had been placed in petrolatum. Carrel deposited vessels, flaps of skin, and periosteum in preservative media and put them in refrigeration immediately after removal. He then examined the tissues by cultivation and transplantation. His work was based on the fact that elemental death or destruction of the living tissue is a slow process.

Albee (23) published one of the first standards for the preservation of homogenous grafts: the graft was to be either immersed in petrolatum or wrapped in sterile petrolatum gauze and placed in storage at a temperature of 4 to 5°C. (39.2 to 41.0°F.). He believed freezing to be undesirable as "the resultant contraction and expansion damage the cellular content of the graft."

Dead bone was transplanted by Leriche (24) (1913) for sarcoma of the upper end of the humerus. The bone was taken from a cadaver and boiled. Union took place but recurrence necessitated an interscapulothoracic intervention in a period of some months.

Groves (25) in 1917 maintained that sufficient success had been attained in the use of chilled homogenous grafts to justify their use in selected cases, if the correction of a defect included all or part of the cartilaginous articular surface of the long bones. Grafts should be taken from a cadaver free from infectious diseases. The graft should be cultured and placed in a sterile receptacle in a refrigerator. If after 24 hours the culture was negative, the graft could be used.

Gallie (26) reported on the use of boiled bone in 1918. In his series of 60 operations in which he used boiled bone, primary union and anatomical and physiological cure were obtained in all cases. In the first of 4 cases reported, boiled plate from the human tibia was implanted. In the second case the feasibility of locking spines together for 2 years by means of boiled bone transplants was demonstrated. Union of the boiled spinal graft to spines by laying down of new cancellous bone upon it, and reestablishment of circulation by ingrowth of blood vessels into empty haversian canals were obtained. The graft was invaded by osteoblasts along the course of the blood vessels. These osteoblasts slowly accomplished absorption of the dead bone and its replacement with new living bone. In the third case a piece of bone was taken from the cortex of the human tibia at autopsy; in the fourth, the radius of a dog was used, with satisfactory results. Gallie held that the use of boiled cadaver bone is as successful as that of autogenous bone where fixation rather than osteogenesis is mainly required.

In a case of a soldier with injury of the knees, reported by Christophe (27) (1923), a homogenous graft of the patella and tendons of the quadriceps and patella were used. Two patellas taken from a cadaver were fixed in alcohol at 80°C. Tendon of the patella was inserted between the patellar tendon and the articular capsule. Union occurred by primary intention, and in 4 weeks the patient was able to return to service. Later, in civil life, flexion was good, and no abnormal symptom was present. Christophe believed this to be a revival of grafts of dead tendons. In another case a cubitus fixed in alcohol for 10 days was grafted in an osseous cubital resection, resulting in consolidation. In a third case the mechanical result was complete in 2 months after the use of cadaver radius preserved in alcohol. He considered that a graft of bone, fixed in alcohol after possible delay following death, furnishes an ideal material.

In 1937 Orell (28) of Stockholm reported on his now well-known "os purum." This is beef bone, from a slaughter house or from amputation specimens, which is composed of a calcium framework of dead bone. Through a physicochemical process, heterogenous beef

than cortical bone and constitutes an excellent material for grafting. When spongiose bone is combined with compact bone the results are better.

Bone preserved in a deep freeze unit for 225 days has been used by Converse and Campbell (39) (1950). At the Hospital for Special Surgery in New York bone has been stored as long as 436 days. During the last two years they have used bone autografts and homografts preserved in a bone bank for plastic surgery in 36 different surgical procedures in 20 cases. The refrigerated homografts were used to restore contour in the frontal area, for saddle-nose deformity, and for repair of the mandible; all being successful. They were observed over periods of 6 to 19 months, and there was no evidence that refrigeration interfered with the fate of the transplanted bone. Homograft and autograft behaved similarly.

During the last 20 months Harmon (40) (1950) carried out 131 transplantations of homogenous bone preserved in deep freeze or in aqueous merthiolate, and 103 transplantations of autogenous bone. The period of longest storage was 2 months in the freezer, and 3 months in merthiolate. He observed no difference in operations with bones preserved for longer periods. Comparison of sequestration rates where autogenous bone has been used with those in similar cases in which bank bone was employed showed a marked advantage for the use of autogenous bone.

Zimbron (41) of Mexico used homogenous bone fragments from a bone bank in 128 patients. He found the freezing process to be superior to preservation by ordinary refrigeration, autoclave, or merthiolate. Cicatrization, tolerance of the graft, and clinical consolidation were observed over a period of 3 months. Zimbron noted that frozen cadaver grafts clinically are equal to autografts or homografts not frozen. Bone grafts have now (1950) been employed in 325 operations, with tolerance in 97.6 per cent, and with clinical consolidation in 91.4 per cent, and from the roentgenographic viewpoint, in 82.8 per cent. The histologic report from biopsies has been reconstituted bone.

Hyatt[3] (42) (1950), of the United States Naval Hospital at Bethesda, pointed out that a major disadvantage of the methods to preserve grafts of homogenous bone is the lack of distinct control of the asepsis of the deposit. Some institutions place reliance solely upon the wound healing of the donor as the criterion for an acceptable bone graft. The bone bank has grown to be established practice for orthopedic surgeons despite the limited indications for the use of frozen bone. Hyatt believes that it is possible for partial protein denaturation to occur in bone as a direct result of freezing. Frozen bone stimulates active bone formation on the part of the host.

Herbert (43) (1951), of the Rheumatism Center of Aix-les-Bains, analyzed 82 cases in which 87 refrigerated grafts (8 homogenous and 7 heterogenous) were used. In patients in whom autografts were combined with homografts no difference in the progress between the two types was observed. Herbert stated that it is now known that there is little difference in the behavior of bone grafts in the body, whether they be autogenous, homogenous, or even heterogenous. The use of homogenous grafts avoids a second wound and loss of bone from other parts of the body, and almost an unlimited amount of bone is available. Large grafts may be inserted, with better results.

In Wilson's use of refrigerated homogenous grafts transplanted in 307 operations the bone was shown to be well tolerated, with

[3] George Hyatt and his colleagues have been using bone and cartilage grafts preserved by quickly freezing and dehydrating the tissues and storing them in vacuum containers. The dehydrated grafts are immersed in saline solution to regain their water content before use as transplants.

duces the necessary supply of local calcium salts for calcification of the callus. The bone is absorbed or disintegrates and the cortical fragments are gradually replaced by living bone cells.

Weaver (35) in 1949 reported on 49 operations in which preserved frozen homogenous bone was used for grafting. The shortest period of storage before use was 3 days, the longest, 308 days. The donor bone was secured at operations such as rib sections and so on, and from fresh cadaver of young adults. The bone was denuded of soft tissue; periosteum was left intact, if possible. The results in a series of operations in which massive homografts were employed for repair of non-union of fractures were not as satisfactory as repairs with autogenous grafts. Weaver believed that there is greater tendency for the massive homogenous graft to "die" and, on the whole, the time of amalgamation has been longer. Homogenous bone must be handled more than autogenous bone, thus creating a greater hazard. Weaver felt that bone from fresh cadavers is just as efficient as that taken from patients.

Stuck and Dandridge (36) (1950) reported on their experience with 60 cases covering a wide variety of conditions in which they used 66 bone grafts preserved in the deep freeze for variable periods prior to use. There was 6.6 per cent failure. Solid bony union occurred in 53 patients within 6 months following the application of refrigerated bone grafts. They concluded that massive cortical grafts of homogenous refrigerated bone are suited mainly for spinal fusions. For ununited fractures homogenous grafts are best used as thin slices of cortical bone placed as barrel-stave grafts about the host bone. Cancellous bone from the bone bank is useful to fill spaces about an ununited fracture, to supplement cortical grafts, and to fill hollow bone defects.

In a report by Le Cocq and his colleagues (37), preserved homogenous bone in massive grafts and small chips was used for transplantation in 74 orthopedic operations upon 72 patients during 15 months. Complications were few, and the wounds healed by primary intention in 50 per cent of the cases.

Sicard and Binet (38) (1950) described the functioning of a reserve of bone grafts at the Beaujon Hospital in France, where material from cadavers and amputated parts obtained at operation are preserved first at $-35°$C. and maintained at $-15°$C. These sources are utilized at an average of one to four weeks. Of 203 operations for various conditions, 196 showed normal results with healing by primary intention, and 7 (lumbosacral grafts) were complicated by local accidents. Sicard and Binet concluded that the homograft preserved by freezing behaves identically like the fresh autograft. Its clinical, roentgenographic and biologic evolution is the same. The homograft dies after transplantation and then becomes revitalized and rehabilitated from the host tissue. At the end of 48 hours of freezing it appears quite normal; at the end of 3 weeks it can still be normal. Such a bone graft transplanted, then recovered by surgical intervention, showed at biopsy very slight lamellae without binding cells, necrotic interlamellar spaces, filaments without recognizable structure, without stained nucleus, and without red globules. Contrariwise, at the end of 8 months the medullary spaces had a normal aspect, the vessels contained blood, the connective tissue was well stained, showing it to be rehabilitated. Sicard and Binet believe that the homogenous graft does not die at the beginning of preservation, and if one utilizes it fairly quickly, it will be found to be in an identical condition as an autogenous graft. If delay of preservation has been long, the graft is a dead bone but it is rehabilitated in the same way from the host tissue.

In the opinion of Sicard and Binet, spongiose bone is revascularized more rapidly

cancellous chip grafting in chronic osteomyelitis. Lloyd-Roberts feels that preserved homogenous chip grafts were not long delayed in comparison with autogenous chips. His impression is that boiled bone is not significantly slower in becoming incorporated. He believes that boiled bone may be employed with confidence when a bone graft is is required for non-union of a fracture, to supplement an intra-articular arthrodesis, or to obliterate a bone cavity or a surgically-produced bone defect.

In more than 125 patients at the Fitzsimmons Army Hospital in whom preserved homogenous bone was used, Taylor and Kessler's observations tend to substantiate the fact that under given circumstances the use of preserved homogenous bone is justified in every way (50).

Raoul-Michel May (51) (1952) has the impression that dead grafts of human bone from cadavers, preserved by cold, have given excellent practical results for some years. It seems that the preserved dead bone homograft behaves in a manner identical with that of the fresh autograft. Its clinical, radiological and biological evolution seems to be the same. But it presents numerous advantages: simplication and shortening of the duration of intervention by suppression of the time of removal, suppression of the take on the patient, which permits less surgical risk, and the suppression of postoperative discomfort to the region of removal, utilization of an unlimited volume of osseous material, especially in children and patients with fragile decalcified or atrophic skeleton, which cannot furnish bone for autograft in satisfactory quantity or quality, finally the possibility of largely using spongiose bone, which is revascularized more rapidly than cortical bone, and which produces a solid mass shortly.

In May's opinion, dead graft of bone has reached a stage of practical realization extremely satisfactory in human species. But unhappily this technique cannot be applied to tissues other than those of a connective nature, where rehabilitation through nonspecific cells cannot replace the physiologic action of the original cells.

Capurro and Pedemonte (52) (1953) reported a case in which they removed the entire femur for hydatid cyst, with periosteum attached, and inserted a femur from a young girl two hours after death by accident. This bone was refrigerated for a short time. The bloods were incompatible. The new femur was inserted with the periosteum intact, and the muscular attachment was sutured. No attempt was made to reconstruct the ligaments of the joints. The femur was immobilized for one month, then the patient was allowed to walk with a plaster cast in place. There were a few degrees of movement at the knee and hip joint. Serial radiograms showed no change in the graft ten months after the operation. The patient walks without pain. No observations were made on the fate of the cartilage. Biopsy ten months after transplantation showed dead cortical bone but medullary bone had been rehabilitated and living bone cells were present in the lacunae.

The whole calcaneus in a boy was removed by Ottolenghi and Petracchi (53) because of chondromyxosarcoma. A calcaneus removed from a cadaver ten days previously and refrigerated was inserted and fixed to the talus by a nail. The tendo Achillis was sutured. A roentgenogram showed fusion of the graft with the talus. The operation was done on August 31, 1948. When the patient was last seen on October 39, 1952, he was working as a truck driver. Another roentgenogram showed increasing resorption of the transplanted bone but the graft was still present. A biopsy in April 1949 showed that the blood vessels had adhered to the host connective tissue between the necrotic bone trabeculae.

Lance (54) (1953) reported on 160 operations of a great variety in which he resorted

successful results in 85 per cent. Healing of cancellous transplants took place more rapidly than with cortical grafts. Wilson (44) holds that transplants preserved for more than one year do not heal as well as those that have been preserved for a shorter period and the failure rate is higher. There was no sepsis except in one case of unsuccessful cancellous chip grafting in chronic osteomyelitis. Wilson feels that homogenous chip grafts were not long delayed in comparison with autogenous chips. His impression is that boiled bone is not significantly slower in becoming incorporated. He believes that boiled bone may be used with confidence when a bone graft is required for non-union of a fracture, to supplement an intra-articular arthrodesis, or to obliterate a bone cavity or a surgically-produced bone defect.

On the basis of his experience with a total of 278 homogenous bone grafts used in operations on 214 patients, Wilson (45) (1951) drew the following conclusions:

(1) With careful technique homogenous bone grafts may be preserved for long periods of time for surgical use.

(2) Such grafts are well tolerated by human tissues and the risk is no greater than when autogenous grafts are used.

(3) The healing of such grafts takes place by a process of invasion, absorption, and replacement similar to that of autogenous bone grafts.

(4) The results obtained are identical with those from the use of autogenous grafts except that in some instances the healing appears to be slower.

(5) The operation of a bone bank is safe and practical.

On Okelberry's service bone is frozen while still moist and is thus preserved in its own fluid in a deep freeze unit (−20°F.). Thirty-six grafting operations were carried out in which refrigerated homogenous bone was used. One infection occurred in a previously-infected compound fracture of the tibia. There was failure of union in another tibial defect; one patient developed pseudarthrosis of the spine. In the other 33 patients the results were good (46).

From the Veterans Administration Hospital at Louisville comes a report by Gordon and Welsh (47) (1951) in which they state a shortage of supply from autopsies and objections to cadaver specimens. It was decided to use bone from legs amputated because of gangrene resulting from arteriosclerosis, diabetes, or frostbite. As much as possible of the femur, tibia, and fibula are removed aseptically, most of the soft parts being stripped away. Bone segments are prepared in 6 to 8 inches in length, and ½ to 1 inch in width. As much as possible of the head and condyles of the long bones are conserved and left attached to the cortex. Strips of bone, freed from marrow, are immersed in glass cylinders containing sterile isotonic saline, for 5 days. In a series of steps the osseous sections are tested and transferred to fresh solutions of different strengths and kept at refrigeration temperature (about 5°C.). Additional cultures are made at monthly intervals and noted. The surgeon is allowed to select the most suitable piece and reports regarding success or failure of operations. No evidence of bacterial contamination and no infection nor sloughing have been reported.

Elliott and Scott (48) (1951) noted that microscopic studies of bone removed from fused neurosurgical patients in whom homogenous bone had been used, showed the graft to be "dead" but surrounded by connective tissue undergoing metaplasia and new-bone formation.

Lloyd-Roberts (49) of St. George's Hospital, London, reported on wide experience with cadaver bone from the tibia and ilium, scraped, boiled (for one-half hour), stored dry in the refrigerator and grafted in humans with various bone conditions. There was no sepsis except in one patient with

bone graft die after transfer, and the osteocytes in preserved and some refrigerated grafts are dead at the time of transplantation, the grafts seem to behave in about the same way. There is not sufficient evidence at this time to state precisely that refrigerated or frozen bone grafts are superior to grafts stored in alcohol and other preservative solutions or heat treated but there is a general tendency among clinicians to use refrigerated or frozen bank bone. Preserved autogenous bone grafts either heat killed, as in cancer patients, or refrigerated are well tolerated by their host tissues and replaced under favorable circumstances by creeping substitution. Their field of use is obviously limited to selected cases where autogenous bone may not be used in the fresh condition at a single operation.

Nature is profligate and generous with her little-understood process of osteogenesis. When autogenous bone grafts with open structure are transplanted, the cells in the graft appear largely to survive and the process of new bone formation merely serves to cement the graft firmly in place. When homogenous bone grafts with dead cells or living cells, which will soon die, are transplanted, the graft structure is absorbed and replaced in kind by new living bone cells and new calcified structure. Thus, creeping substitution is used when necessary to replace homogenous bone grafts and possibly also thick autogenous cortical grafts, in which the cells fail to survive.

From a scientific standpoint it is difficult to compare the clinical usefulness of bone homografts with that of bone autografts *but the available evidence indicates that autogenous bone grafts are preferable when they can be obtained*. Certainly the healing is more rapid with autogenous bone grafts, and this is a very important factor.

The homogenous bone graft occupies about the same position in bone grafting as does homogenous cartilage in the field of cartilage grafting. *Both are inferior to autogenous grafts.*

Bank bone undoubtedly has a limited field of usefulness in selected cases where it is not feasible to use autogenous bone. When bank bone is available, however, there is a tendency to use it in patients where autogenous bone could be utilized to better advantage.

The author emphasizes that autogenous grafts with living cells are always the best type of grafting material in humans; this applies even to corneal and lens transplants. Fresh or preserved homogenous grafts are used with different degrees of success for different types of tissue when it is not wise or practical to use the patient's own tissues.

With homogenous bone grafts in contact with bone, the host tissues may absorb and replace the foreign graft with autogenous bone through creeping substitution. Contrariwise, other tissues such as homogenous fat grafts are completely absorbed and not replaced as the same type of tissue. Homogenous bone grafts, either fresh or preserved, are absorbed unless the grafts are in contact with bone and preferably subjected to functional forces of stress and strain. Immobilization after grafting is also an important factor in the replacement of homogenous bone grafts as bone.

Although there is still some disagreement regarding the survival time of the cells in fresh homogenous bone grafts, all investigators believe that the cells eventually die and are replaced by new cells from the host bone, from the host periosteum, or the surrounding soft tissue. In general, it is advantageous to transplant both fresh and preserved homogenous bone grafts with the periosteum removed.

In comparing the behavior of the fresh bone homografts with that of other fresh tissue homografts, it is interesting to note that the cells in free grafts of all kinds of tissues which have a blood vessel system may

to the bone bank at St. Francis Hospital, Wichita, Kansas, begun in early 1950. The sources of bone are cadavers, amputations, ribs removed at thoracotomy, ilium, clavicle, patella, ulna, and boiled bone. The first cadaver bone, which had been in the freezer for 18 months, was used with clinical results as good as those from any other bone. There was no apparent harm to the bone from a thawing and refreezing procedure. The homogenous bank bone has accomplished its intended function as well as autogenous bone. The orthopedic surgeons have been able to complete certain procedures successfully with ease that would have been impossible or done at great risk and with difficulty without bank bone.

Reeves (55) (1953) reported that the wound infection rate following transplantation of frozen homogenous grafts is about 2 per cent, but is proportionately higher in those series where grafts are used in compound wounds and osteomyelitic cavities. He noted that the process of freezing does not keep the bone cells alive but merely preserves them in a fresh state. Homogenous bone serves as a framework to guide invading elements of the host, also a catalytic function in that its presence promotes an osteogenic reaction, and as a local supply of calcium. In the microscopic examination of sections, dead spicules were surrounded by connective-tissue cells, with osteogenesis taking place by metaplasia. Bone being absorbed, its place was taken by living cells.

During four years Sicard and Mouly (56) (1953) have carried out 592 preserved homogenous bone transplantations from cadavers. The preservation was by refrigeration at −18°C. after freezing at −35°C. Their clinical results were verified by their experimental findings (see Chapter 16, page 170). They concluded that homogenous grafts preserved by refrigeration are of indisputable value on condition that the surgeon complies with the exigencies of the method. The homograft is thus capable of replacing the fresh autograft but it is necessary to know that its osteogenic activity is slower and a longer immobilization is required.

Abbott claimed to have been the first to describe the freezing of cranial bone for transplanting an autograft or homograft on the skull. This work was begun in January 1950 in a case of decompression after removal of a left frontal parasagittal meningioma when acute edema developed in the left frontal lobe. The autogenous bone preserved in the deep freeze at −10°F. ("quick frozen") for 16 days was replaced at its former site. The take was normal, and there was no evidence of serious shrinkage or loss of calcium content after two years.

Abbott (57) (1953) reported on a group of four autogenous cranial grafts replaced in former sites, a group of four homogenous cranial grafts, and a third group of homocranial grafts of multiple frozen discs used for fusion of the lumbar vertebral bodies after removal of the major portion of the intervertebral disc. He believes that the time interval since these operations has not been long enough to evaluate the method. In recent cases the bone has been dipped in, or sprinkled with, an aqueous solution of penicillin or streptomycin. It is also his policy to place penicillin and streptomycin solution in the cranial bone-grafted wound. Some of the cranial bone grafts, he stated, may have shrunken a little but not significantly from a clinical viewpoint. Abbott concluded that freezing of cranial bone when available is a safe, satisfactory and desirable method; he considers it a normal body tissue for grafting in the skull or spine.

SUMMARY COMMENT ON FRESH AND PRESERVED HOMOGENOUS BONE GRAFTS

It appears that homogenous bone grafts have a definite place in bone-graft surgery. Although the osteocytes in fresh homogenous

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survive for about eight days to a few weeks following transplantation.[4] The cells in fresh homografts of cornea, cartilage, and lens, which do not have a blood vessel system, survive for much longer periods of time after transplantation. The epidermis of the skin appears to be an exception since it also has no vascular system but transplants usually include some dermis, which does have a vascular system. The epidermis also lacks the protective mucoprotein matrix present in cornea, cartilage, and lens.

Hyatt (58) comments that the host acceptability and utilization of autogenous tissue surpass any known substitute; therefore, *when available to the clinician the autogenous tissue remains the graft of choice.* The "bone bank" furthers the ends of the stored tissue principle in the belief that there is a definite, although limited place, for homografts when they are used by expert surgical technicians who temper skill with considered and mature judgment.

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[4] This statement does not apply to the cells in organ or gland homotransplants such as the kidney, where the host artery and vein are joined to the graft artery and vein. In this type of homotransplantation the graft receives an immediate total transfusion of host blood, and kidneys transplanted in this way have secreted urine up to 90 days after transfer.

CHAPTER 19

Heterogenous Bone Transplants in Humans

It is rather difficult to evaluate the status of bone heterografts on the basis of published factual data. One finds in the literature far more attempts to use such grafting material than might be expected and many reported successes.

REVIEW OF LITERATURE ON HETEROGENOUS BONE TRANSPLANTS

In 1889 Senn reported 4 successful cases of decalcified ox bone transplantations (1). Miller (2) in 1890 used ox bone chips in a patient with cystic lesion of the upper tibia. A piece of ox rib was scraped, decalcified, put in carbolic lotion for 48 hours, and then cut into small pieces. The cavity in the head of the tibia was filled with these ox shavings. Having an insufficient amount of the ox bone, Miller added some autogenous bone. There was some postoperative drainage, which stopped only when the autogenous calcified bone was extruded.

In a tailor with osteomyelitis of the first phalanx of the index finger, Kronacher (3) used a cortical graft from a calf's leg. The graft was freed of marrow, boiled in soda solution and preserved in carboalcohol solution. There was no reaction; the foreign body bone was well tolerated and solidly set. After a year active movement was 150° and extension complete. The patient could sew again. Radiographically, new bone proliferation surrounded the calf bone. The entire implanted bone was sharply outlined against its surroundings. After this case Kronacher used the same form of heteroplastic graft in spina ventosa resections in two small children, apparently with good results.

Kuettner (4) in 1913 reported a case of a child with congenital absence of the fibula in whom he grafted the fibula of a Java monkey with its epiphysis. Radiograms taken over a period of several years showed permanent preservation of the outline of the grafted bone.

König (5) in 1913 used ivory intramedullary pegs and periosteal ivory plates for fixation in fractures. Encouraged by the results, he had ivory models made of various parts of bone to replace parts lost by injury or disease, e.g., half the lower end of the humerus in one case and half the lower jaw in two cases. In several of these patients it was necessary to remove the foreign body after a period of several months or years. In a woman, aged 62, half the mandible was replaced by an ivory prosthesis, with a perfectly satisfactory result two years after the operation.

Magnusen (6) (1913) reported that ivory could be inserted into bone with very little force, and after 24 hours it absorbs enough moisture to fit. When in close contact with

55. Reeves, H. G., Jr.: Bone bank. Maryland M. J., **2:** 495, 1953.
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Employing cow's horn, Fowler (1934) noted that nine fractures united promptly with superabundance of callus and bony union, without complications. The horn was gradually absorbed (17). In search for an explanation of the apparent stimulating effect of callus formation, he reviewed the literature on the subject. In 30 cases including his own where horn had been used for internal fixation of fractures, it seemed to have been ideal material for strength, elasticity, absorbability and ease of sterilization. It appeared to be non-irritating, mildly bactericidal and stimulating to callus growth (18).

Calvé (19) (1935) employed heterogenous spongiose tissue from a young calf for vertebral osteosyntheses, tuberculous arthritis, and arthritis in 15 operations. The osseous substance was admirably tolerated, and the clinical results were excellent. The fragments of calf tissue in small bags were preserved in ether. At the time of use the fragments were bathed carefully in artificial sterilized serum. In a case of pseudarthrosis of the tibia grafted with ox bone Leriche (20) (1935) confirmed the complete cure 9 years after operation. He considered the permeable factor of bone most important.

The use of heteroplastic grafts was fully justified in a number of cases of pseudarthrosis and also in recent fractures in which the use of metal was inconvenient and difficult, as reported by Danis (21) (1936). From actual experience he was favorably impressed with the use of this grafting material. When the wound remains aseptic, the ox bone is very acceptable. But infection makes it untolerated more quickly than when fresh human bone is used. He was not able to judge the late results of his heteroplastic transplants. He raised the question whether os purum will give proof of revitalization (modifying form and intimate structure) as has been observed in autoplasties, which always respond to the mechanical needs of the skeletal environment.

Carrell (22) (1936) advocated the use of prepared cow's horn as material for internal fixation in bone surgery and used it in 40 cases. He believed it has adequate strength and produces abundant callus formation without reaction.

Orell's os purum and os novum have been previously discussed under preserved homogenous bone grafts, for he used both ox bone and human cadaver bone prepared by a definite process before being finally washed, dried and sterilized by boiling. He used these materials with considerable success. A number of other surgeons, incited by his favorable report, undertook further experiments to prove the worth of these grafting materials (23).

For mechanical fixation Zygmunt (24) (1937) advocated the employment of heterotransplants which, he believed, were resorbed and transformed like autoplastic material. The implantation of a graft induces hyperemia, which accelerates the separation of diseased tissue and stimulates the osteogenic proliferation. Beef bone and bird bone were used for osteosyntheses, shelf operations, fractures, pseudarthroses and other conditions.

In a case of a boy of 18, reported by Groves, ivory from a walrus tusk was used to fill a large cavity in the femur in 1922. In 1938 the ivory had not undergone any change. In a boy of 10, with fibrocystic disease of the right humerus, beef bone was used to reconstruct the humerus. The length and general structure were the same as those of the unaffected humerus. In this boy, who became an athlete, ghostly shadows of beef bone could be traced in the midst of human tissues 10 years later. In a woman with a fracture of the neck of the femur, Groves constructed a bone peg made from a stag's antler. She could walk well a year later. In radiographs taken 3 years later the bone

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CHAPTER 20

Clinical Use of Bone Grafts

A questionnaire was sent by the author to a selected group of orthopedists, plastic surgeons and neurosurgeons, asking for an opinion regarding the merits of the different types of bone grafts for clinical use.

QUESTIONNAIRE OPINIONS ON TYPES OF BONE GRAFTS

All of these surgeons *preferred living autogenous bone* instead of homogenous or heterogenous bone whenever the first type was available. Most authorities agreed that homogenous bone, either living or dead, was often replaced by creeping substitution from the living host bone or its periosteum. A few did not use homogenous bone in any circumstance, and none used heterogenous bone grafts. All agreed that the presence of periosteum on autogenous grafts is not necessary for survival of the graft structure, although Sterling Bunnell, Kazanjian and Henry Kessler believe that it is helpful.

Robert Ivy does not think that homogenous bone grafts survive transplantation, but it is possible that they stimulate the living bone with which they are in contact to produce new bone. On the other hand, he is sure that living autogenous bone transplants do survive as such. Ivy further states that he has yet to see a report of a successful homogenous bone graft restoring continuity of a movable long bone in the human. "Bone banks" may be useful as a source of material for filling bone cavities but, he believes, there is a definite limit to their usefulness. Kazanjian feels that the supply of autogenous bone is so abundant and the results so dependable that there is little need to use more undependable materials.

George M. Wyburn and his colleagues in plastic surgery at the University of Glasgow favor the use of autogenous bone grafts. The ilium is preferred as a donor site in most instances. Regarding the results of experimental work Wyburn considers it very doubtful that the presence of periosteum favors the survival of autogenous bone grafts.

Rainsford Mowlem uses only autogenous bone grafts and he believes that they tend to survive and grow under favorable circumstances. He emphasizes that bone is a tissue which is designed to withstand stress and strain. If it is grafted in an area where these factors are not present, its initial survival may be satisfactory; but like the excess callus formed around a fracture it is, unless subjected to stress and strain, ultimately removed and its removal is an expression of perfectly normal body function.

Frank E. Stinchfield also doubts that the periosteum adds or detracts from the usefulness of a graft. If the graft is autogenous and the cells expected to remain permanently

viable, then the periosteal cells (fibrocytes) may add a greater volume of cells available

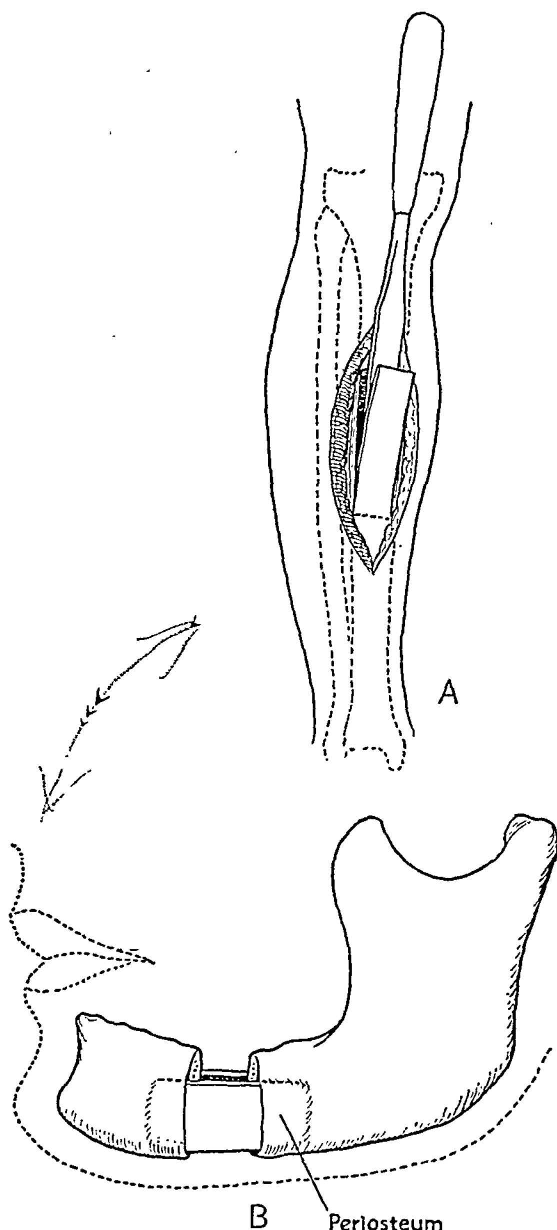


FIG. 84. The osteoperiosteal graft.

A. With a broad, flat chisel the thin shaving of bone with its periosteum is taken from the anteromedial aspect of the tibia.

B. The two pieces of the graft have been placed in contact with the freshened bone surface, one graft on the inner surface of the mandible, the other on the outer surface. The ends of the graft are placed beneath the periosteum of the mandible. From *Principles and Practice of Plastic Surgery*, Arthur J. Barsky. Baltimore: The Williams & Wilkins Co., 1950.

for differentiation into bone-forming cells. If the graft is homogenous the cells will fail to survive transplantation anyway.

The general opinion of orthopedists is expressed by Toufick Nicola when he states that he also prefers fresh autogenous bone grafts. Homogenous bone is replaced by the host bone but the process takes longer than when autogenous bone is used.

Some neurosurgeons prefer bone cranioplasty to artificial substitutes such as tantalum plates, while others use tantalum plates entirely. Opinions differ as to whether the skull bone will form a bony union with the adjacent host skull bone. Francis Grant is certain that this does occur because he has seen fracture lines from external violence run right through a defect repaired by cranioplasty. Contrariwise, Gilbert Horrax of the Lahey Clinic states that skull bone grafts do not form a bony union with the adjacent host skull bone but rather a fibrous union. Horrax believes that the graft does not persist as living tissue but rather is either wholly or partially absorbed and replaced by cells from the host bone. Frequently absorption is incomplete and consequently islands of absorption and replacement are observed.

In discussing a paper by Francis Grant and Norcross on cranioplasty, Howard Naffziger reports that he followed the condition of one patient who had retained a cranioplasty for 22 years. In this case a tremendous loss of bone from the frontal region was repaired with strips of autogenous osteoperiosteal grafts from the tibia, which gave an excellent cosmetic result. In roentgenograms the grafts seemed to be unchanged for some 5 or 6 years, and then successive films showed that the strips of bone were becoming less and less dense and finally after 20 years they could no longer be seen; the cosmetic result, however, was almost as satisfactory as in the beginning but the graft seemed to have been replaced by a very heavy fibrous covering.

Neurosurgeons use dead autogenous skull

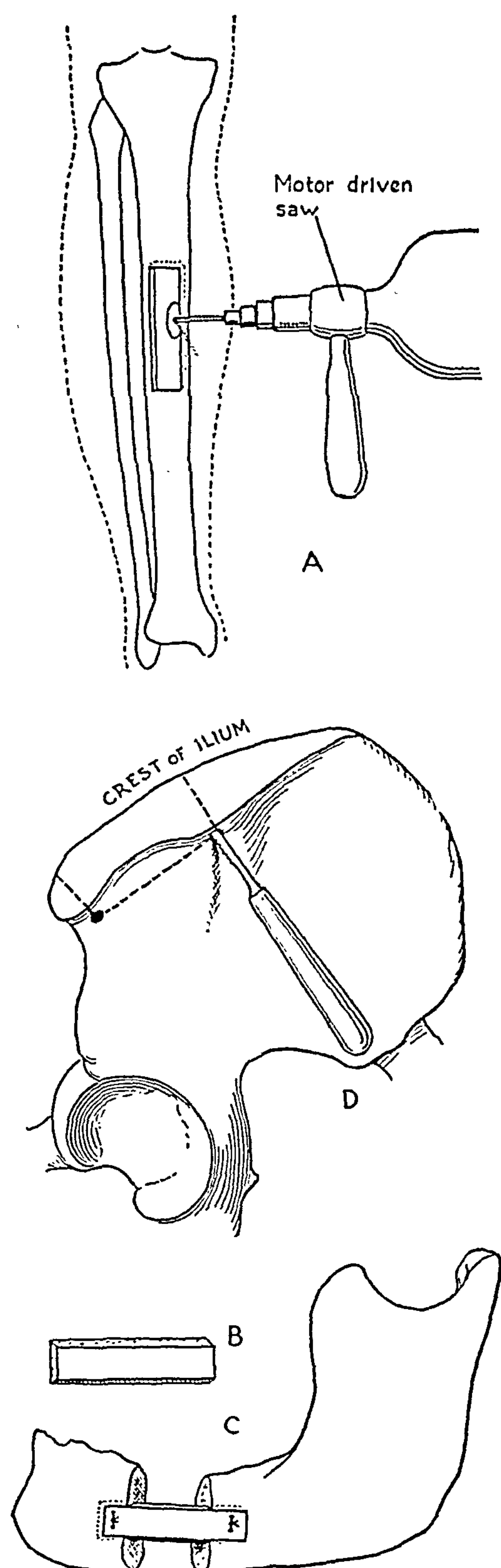


FIG. 85. Bone grafts.

A. Cutting a bone graft from the anteromedial aspect of the tibia.

B. The graft itself is wider on its deeper surface.

C. The graft mortised and wired into place. This can be done easily if the graft is not too long. Otherwise manipulation of the fragments is difficult.

D. Extensive grafts may be taken from the crest of the ilium. From Principles and Practice of Plastic Surgery, Arthur J. Barsky. Baltimore: The Williams & Wilkins Co., 1950.

bone grafts occasionally in cancer patients, when the bone which has been removed is boiled to destroy all cancer cells and then reinserted in the bony defect. There is no conclusive evidence regarding the fate of these dead autogenous bone grafts.

Reed Dingman prefers iliac bone with cortex on one surface for correction of defects in the skull. Although the grafts remain less opaque than the surrounding skull bone, the contour and function are excellent and the grafts appear to form osseous union with the host skull bone. Herbert Conway also prefers iliac bone grafts and he includes the outer cortical layer with the cancellous bone.

The author is in accord with the opinions expressed in the questionnaire regarding the superiority of fresh autogenous bone grafts to those of the homogenous variety. The only homogenous human tissue cells which are known to survive in fresh homografts for longer than from eight days to a few weeks are those in cartilage, in the connective-tissue part of the cornea, and possibly the lens. This survival time is certainly limited in cartilage homografts and possibly also in corneal homografts. Experimental work has demonstrated that autogenous bone grafts heal more rapidly than homografts, and there is also evidence that many of the cells in bone autografts survive transplantation and maintain the specific calcified structure of the graft under normal conditions of functional activity. *One should therefore consider autogenous bone as the best grafting material and use homogenous bone only as a second choice when it is not wise to use the patient's own bone.* The bone bank is serviceable in selected cases but there is a tendency, especially among orthopedists, to

transplant homogenous bank bone in many patients in whom autogenous bone could be utilized to better purpose. If the bone bank were not so readily available they would use autogenous grafts more frequently.

CHOICE OF CANCELLOUS AND CORTICAL BONE

The opinions expressed in the questionnaire regarding the relative merits of cancellous and cortical bone grafts were in general agreement. Medullary bone was favored where quick healing was desired and for filling cavities in bone. Medullary bone with cortex on one surface of the graft was deemed desirable for additional strength and moderately rapid healing, and compact cortical bone grafts were selected when considerable strength was required and the patient could be immobilized for a long period of time because of the slower healing.

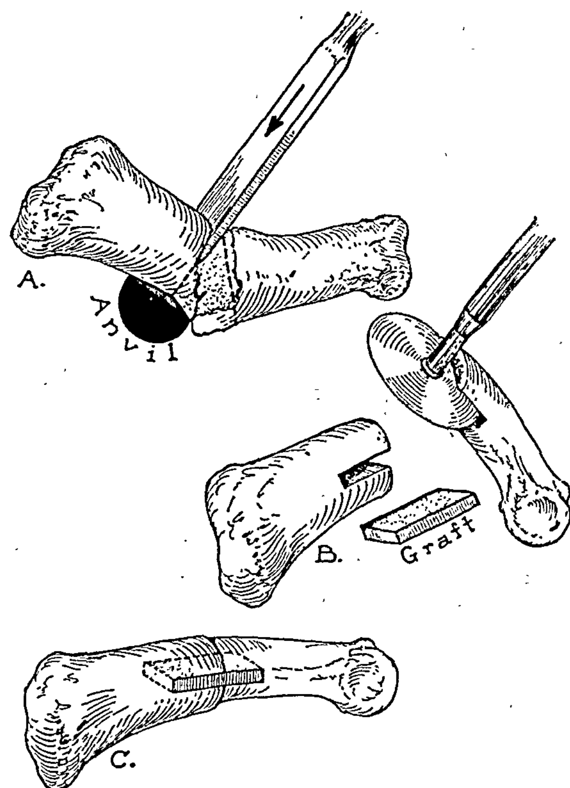
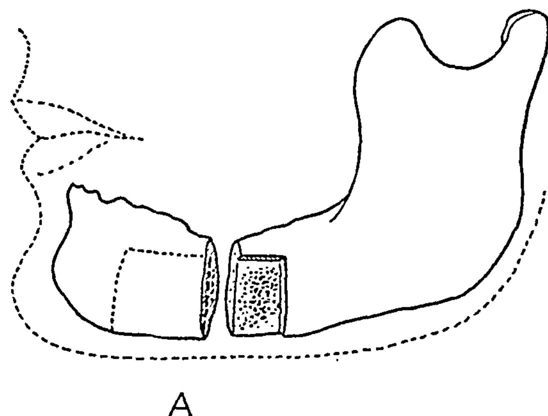


FIG. 87. Bone carpentry. A small key bone graft and a good fit assure against non-union. The point of an anvil is held under the phalanx to facilitate carving with an osteotome. A bone graft may be easily obtained by chiseling off a chip from the ulna of the same arm below the elbow. From Surgery of the Hand, Sterling Bunnell. Philadelphia: J. B. Lippincott Co., 1944.



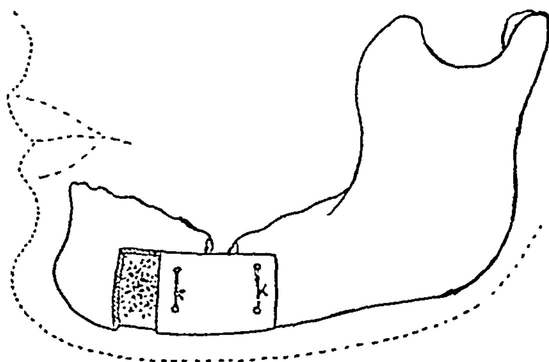


FIG. 86. Sliding bone graft to the mandible.
A. The proximal bone fragment has been prepared to receive the graft which is outlined on the distal fragment.
B. The graft has been slid over into position and wired. From Principles and Practice of Plastic Surgery, Arthur J. Barsky. Baltimore: The Williams & Wilkins Co., 1950.

Most of the plastic surgeons did not favor the use of cortical bone grafts alone under any circumstances occurring in their field of work, whereas the majority of orthopedists used cortical bone grafts in selected cases.

Blocker removes all cortex from the spongy bone of iliac grafts and cuts and shapes them as required by the individual patient. Cancellous bone, in his opinion, develops strength and evidence of firm bony union within the period of 8 to 14 weeks.

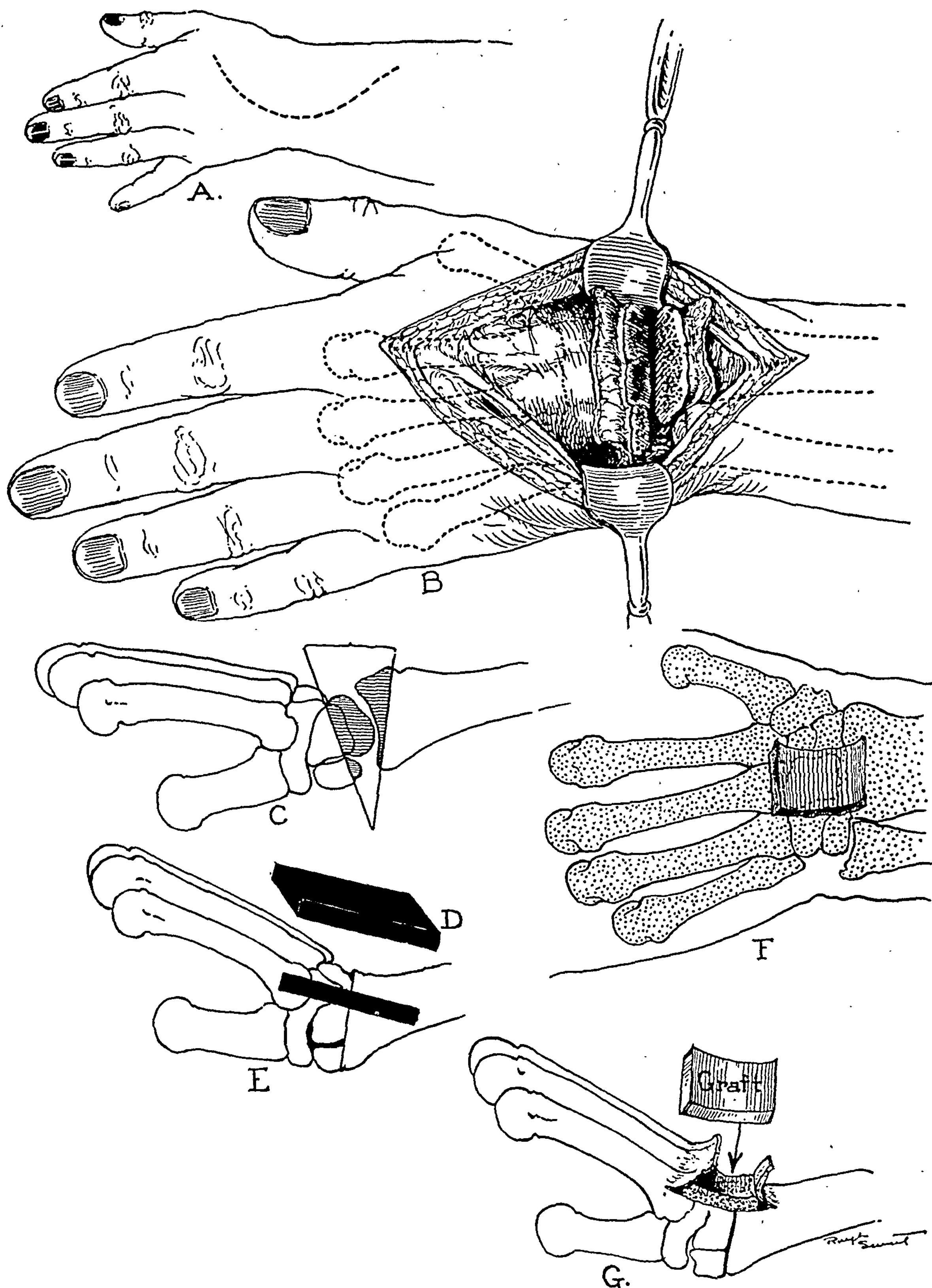


Fig. 88. Arthrodesis of the wrist joint with preservation of the radio-ulnar joint.

A. Incision placed to spare branches of radial nerve and not to overlie the bone graft.

B. Through an H-shaped cut in the joint capsule the latter is peeled back each way with a layer of bone.

C, D, and E. If growth has been attained and stability is desired a wedge resection is done and a bone graft is placed in the central portion of the bones through the epiphyseal line.

F and G. Method used when epiphyseal plate is still active. Spongy graft from ilium is placed rather superficially and the capsule of the joint closed over it.

From Surgery of the Hand, Sterling Bunnell. Philadelphia: J. B. Lippincott Co., 1944

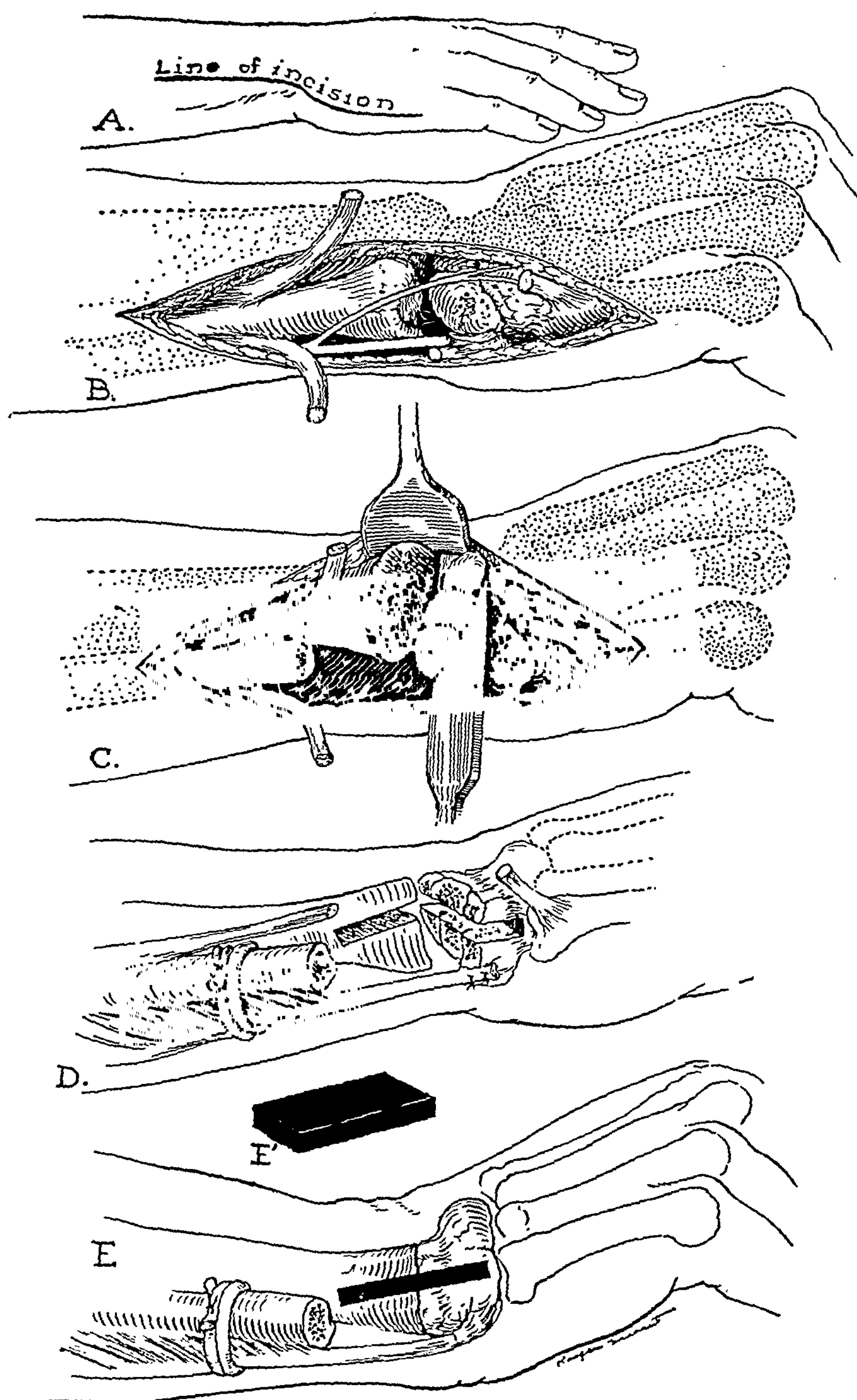


Fig. 89. Arthrodesis when the radio-ulnar joint participates in the ankylosis.

A. The joint is opened by the Smith-Petersen approach.

B. Tendons of the two carpi ulnari severed, and dorsal branch of ulnar nerve retracted.

C. Head of ulna is excised and joint denuded so it fits together at 30° of dorsiflexion.

D. The ulna, now free for pronation and supination, is lashed to the flexor ulnaris tendon to prevent displacement.

E. Bone graft from tibia or iliac crest is placed.

From Surgery of the Hand, Sterling Bunnell. Philadelphia: J. B. Lippincott Co., 1944.

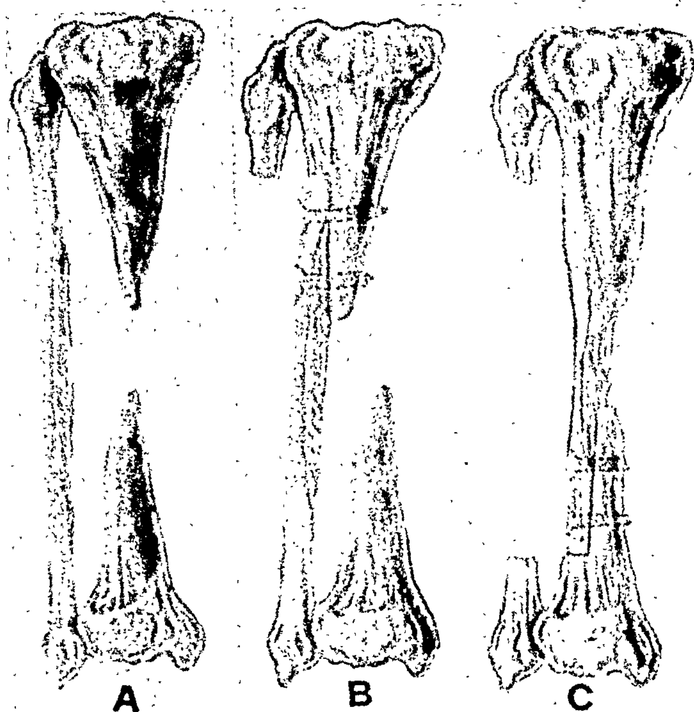


FIG. 90. Transference of fibula to replace defect in tibia as recommended by Huntington.
A. Defect of tibia.
B. Upper end of fibula grafted to proximal fragment of tibia.
C. After solid union proximally, distal end of fibula transplanted.
From Plastic and Reconstructive Surgery, Earl C. Padgett and Kathryn L. Stephenson. Springfield, Ill.: Charles C Thomas, 1948.

The choice of a donor site for cancellous bone and for cancellous bone with cortex on one surface was the ilium. Some authorities felt that rib bone was equally desirable but all agreed that the tibia was the best donor site for compact cortical bone.

The author would add the nasal septum, nasal bones, and turbinates as good donor sites for small bone grafts because they will retain their general size and calcified structure when transplanted in soft tissues. In one patient a hyoid bone transplanted in muscle can be palpated five years after transfer.[1] One speculates as to the survival capacity of skull bone and other facial bones in various soft tissue sites.

INDICATIONS FOR THE USE OF BONE GRAFTS

The various surgical conditions for which bone grafts are employed are so well known that it does not seem necessary to enumerate them in this book.

It is generally recognized, for instance, that bone grafts from the ribs, tibia, and ilium must be in contact with bone in order to retain their calcified structure, and that they must be immobilized in order to form a bony union with the host bone. When movement is present after transfer the graft is apt to form a fibrous union, and in such cases the calcified structure of the graft will disappear or the graft will form a sequestrum and be extruded. A possible exception to this statement is the reported fact that rib and iliac bone grafts transplanted in the subcutaneous tissues of the nose to support a saddle deformity tend to retain their calcified matrix when not in actual bony contact with the nasal bones. Thus, the nasal tissues constitute a favorable transplantation site.

The use of cancellous bone chips around the fractured ends of bone and to fill bone cavities is an old procedure, which is still practiced by many surgeons. The results of cancellous bone chips used to fill depressions over the malar bone and depressions in the skull have been disappointing in the author's experience presumably because these grafts are not subjected to mechanical stress and strain following transfer. They do serve a

[1] Case of Dr. Edgar Cardwell, Newark, N. J.

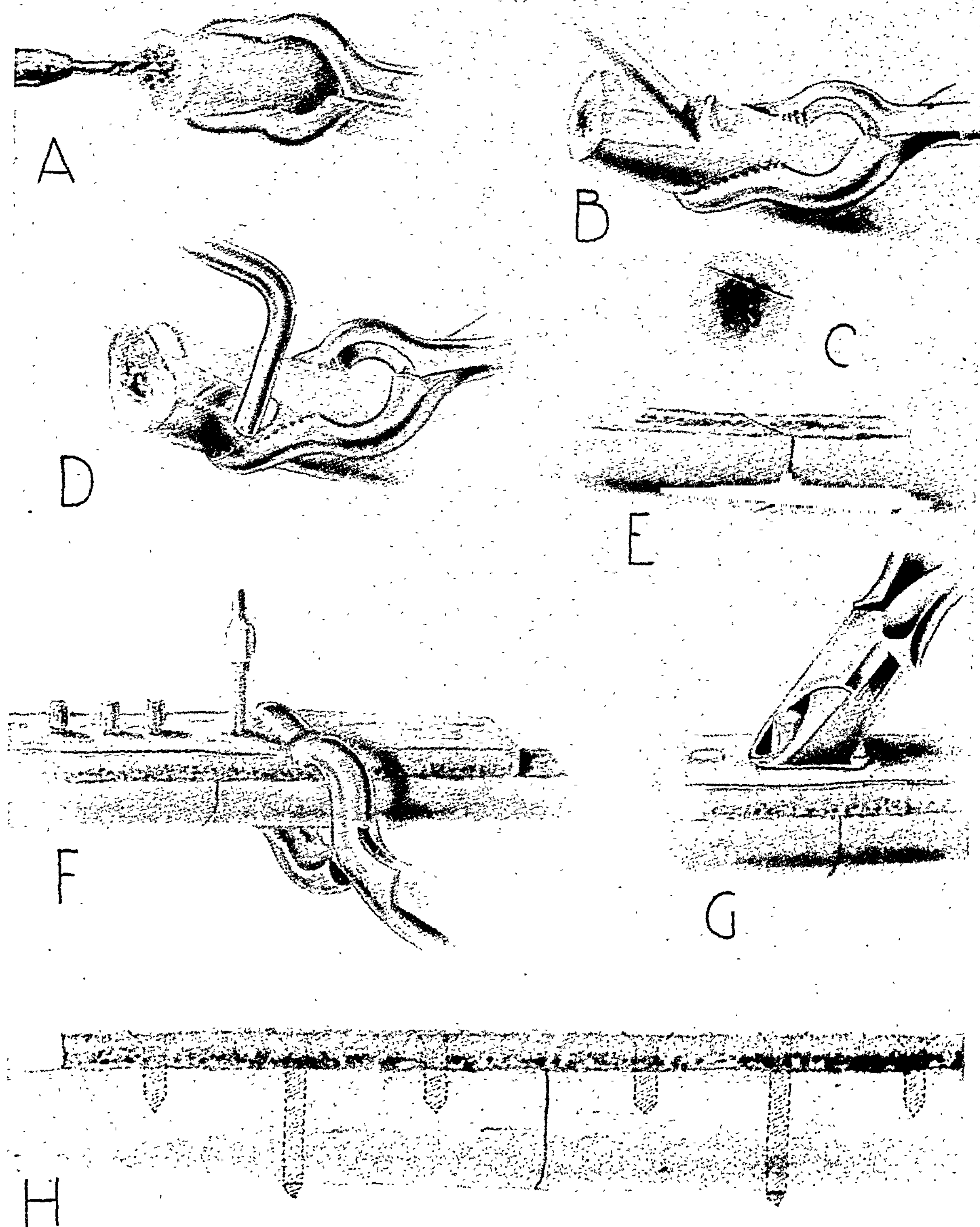


Fig. 91. Technique of massive onlay graft. Campbell prefers an interosseus graft inserted through drill holes in the medulla.

A. The medulla pierced.

B. Round surface of the bone is flattened.

C and D. The ends of the bone are freshened.

E. The freshened ends are approximated.

F. A bone graft is removed from the opposite cortex of the tibia. Drill holes are made in it. Campbell prefers bone pegs, Henderson, beef bone screws and Keys uses metal screws.

G. The tops of the bone screws are cut off flush with the surface of the bone graft.

H. Campbell replaces the bony fragments that were cut off from the top of the bone to flatten it about the side of the fracture.

From Plastic and Reconstructive Surgery, Earl C. Padgett and Kathryn L. Stephenson. Springfield, Ill.: Charles C Thomas, 1948.

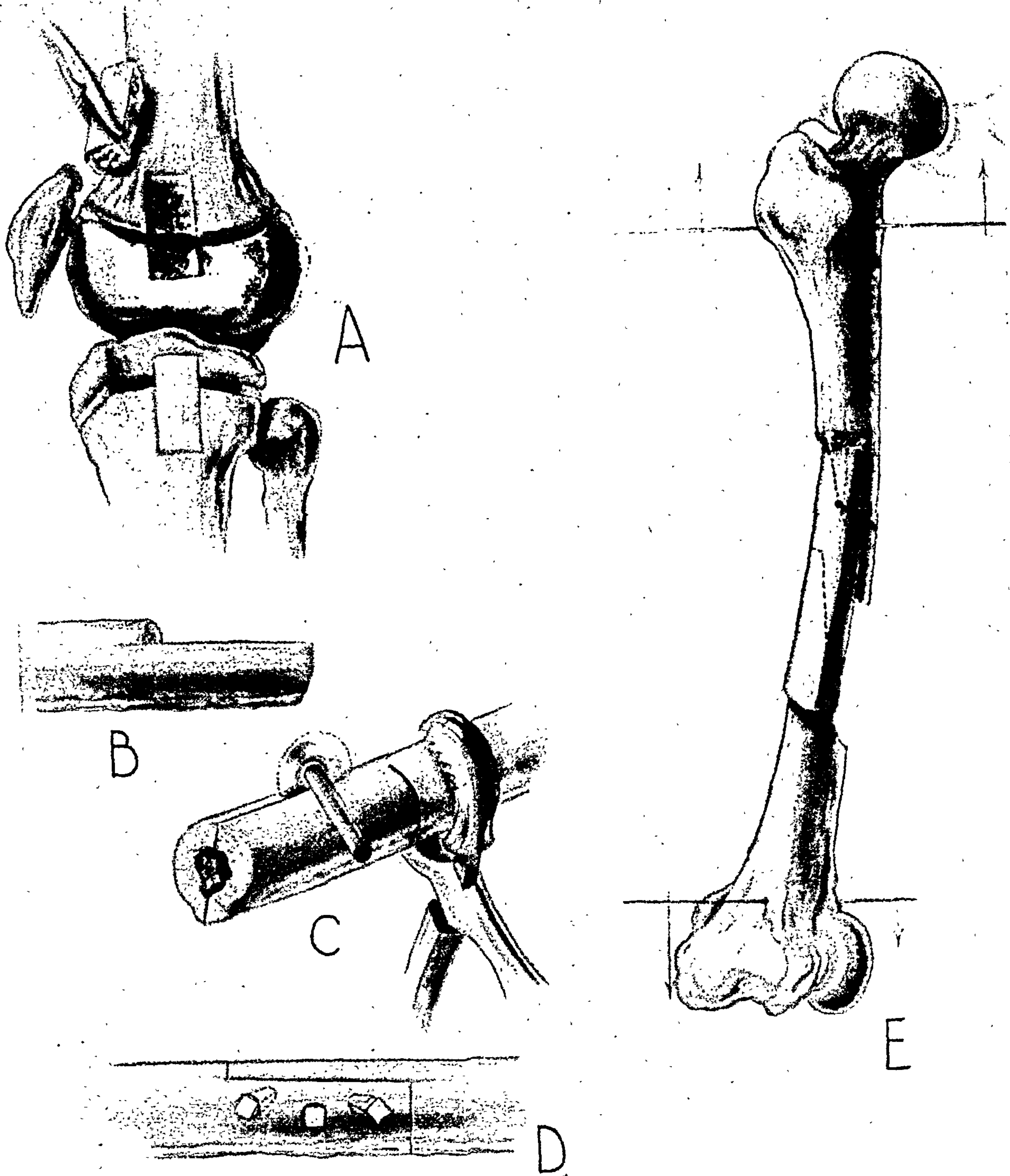


Fig. 92. A. Phemister's technique of epiphyseal arthroplasty for shortening of the leg.

B, C and D. Method of shortening the femur by a stop cut of the desired length. The stop cut is cut with a saw and the fragments are fixed with bone pegs.

E. Lengthening of the femur after Compere. A diagonal osteotomy is made. A tibial onlay graft is fixed to the upper fragments. Two pins are placed through the femur, one in the trochanter region and one in the condylar region. The lower fragments are then pulled down. The tibial bone is shown in place as onlay graft before and after lengthening.

From Plastic and Reconstructive Surgery, Earl C. Padgett and Kathryn L. Stephenson. .: Charles C Thomas, 1948.

normal function in reestablishing contour, and they are in contact with bone but this is often not sufficient to satisfy them. It appears that cartilage as single segments or in diced or shaved form retains its general bulk and structure in skull defects or as a simple onlay graft better than bone chips or single segments of bone in similar locations. This statement applies only to rib, tibial and iliac bone grafts; septal, nasal and turbinate bone grafts act like cartilage grafts and are not affected by functional use.

Bone grafts from the ribs, tibia, and ilium have an accepted field of usefulness in bridging any defect in the long bones, jaw, or spine. Here they are subjected to stress and strain and tend to retain their calcified matrix. They are utilized to immobilize the movable ends of fractured long bones, in spinal fusion, and for fusion in the wrist and ankle. Bone dust and bone chips are often sprinkled over the grafts to hasten osteogenesis and to provide a stronger union.

Rib bone with its attached costal cartilage is sometimes used when one entire side of the jaw bone is absent including the condyle, according to Brown and Fryer (1). In such cases a section of the sixth and seventh rib may be removed together with a portion of the cartilage. The rib bone is immobilized in contact with the host bone in the chin region and the cartilage end is free in the glenoid region. This type of graft often provides improved function. The angulated shape of the composite graft is frequently quite normal for the contour of the jaw, and the rib bone tends to retain its calcified structure if it obtains bony union with the host bone anteriorly. A biopsy removed from a graft of this type by the author two and a half years after transplantation showed normal living bone with normal appearing osteocytes. The structure of the graft, however, had become denser and resembled compact bone.

Septal, nasal and turbinate bone grafts are useful in filling out small depressions in the nose and for establishing a more normal appearance in receding chin.

Iliac and rib bone grafts are used by some plastic surgeons to support saddle depressions in the nose; this is an accepted procedure. The author believes, however, that a more refined result can be obtained by using cartilage as a grafting substance. By refined result one means a nose which is more normal in appearance and does not look as though it had been operated upon. Obviously the results of either bone or cartilage grafts *depend on the experience and skill of the operator*; either graft can be handled well or poorly.

REFERENCE

1. Brown, James Barrett, and Fryer, Minot P.: Bone grafts for large gaps in mandible. Am. J. Surg., 80: 401, 1953.

PART IV

Fascia and Tendon

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CHAPTER 21

Structure of Fascia and Tendon

Fascia and tendon, which are dense connective tissue, take their origins from the same mother mesenchyme which produces cartilage, bone, and so many other tissues. If one removed the gelatinous matrix of hyaline cartilage and the calcified matrix of bone so that the collagenous fibers could be visualized, both tissues would resemble dense connective tissue. In a similar way, if the Meibomian glands were absent in the tarsus, which is dense connective tissue, the tarsal plate would closely resemble fibrocartilage.

Connective tissue binds and retains the tissues and organs in a unit. The degree of binding varies from that of the loose connective tissue around organs and beneath the skin, the cushioning effect of fatty tissue, the yielding support of cartilage, the rigid framework of bone, and the firm connections of deep fascia and tendon.

Loose connective tissue also provides pathways through which blood vessels, lymphatics and nerves gain access to the tissues and organs.

Cowdry (1) has emphasized that integration of the body by connection is only part of the story. *The connective-tissue system provides also for separation.* Ball and socket joints afford examples of both connection and separation. The bones are connected by a capsule but the moving surfaces are separated by a thin fluid lubricant and coated by special friction-reducing tissue. Organs within the body are separated by capsules which keep them apart as units from the surrounding tissues.

The cells and cell groups in each organ separated by connective tissue have developed specific capabilities and the kind of local environment required for the performance of special duties. Even in the cell itself it is the separation and localization of reactions which make life possible. The separation of nuclear from cytoplasmic material by the development of a nuclear membrane is fundamental. Within both nucleus and cytoplasm there is likewise marked localization on the surface of things visible and invisible. In effect, separation of the body into special compartments by connective-tissue partitions, and of the cell into distinct areas by other membranes and by the presence of substances so different physically that they develop interfaces, this is every bit as necessary for vital activities as the space arrangement in a manufacturing plant. If the barriers were broken down and everything mixed together in the body, the cell, or the factory, nothing useful could be done.

Movement between one structure and another in the body is facilitated by the presence of very loose areolar tissue; this same modification of connective tissue is shown in the formation of tendon sheaths

spaces. Unless drainage or relaxation incisions are made through the inelastic fascia, this pressure may give rise to paralysis of nerves and necrosis of muscles, as in Volkmann's contracture of the forearm.

The loose areolar tissue covering the outer surface of the fascia lata and other deep fasciae is similar to paratenon and may be used as a free graft to provide slippery gliding surfaces for adherent tendons (4).

Cells

The fibroblasts, which are the parenchymal cells in fascia, are scattered rather sparsely between the dense bundles of collagenous fibers. Thus, the intercellular substance is the prominent feature of fascia, as is true also of tendon.

The fibroblasts arise from mesenchymal cells and quantities of collagen soon appear around them. This collagen differentiates into collagenous fibers which are arranged in the different types of dense fascia according to forces of stress and strain. The fibroblasts in dense fascia are rather thin flat cells, so that in profile they appear spindle-shaped. The nucleus is large and oval, and the cytoplasm is fairly clear excepting for fine fat droplets. The cells appear to be squeezed into their flattened shape by the dense collagenous fiber bundles which surround them. The fibroblasts in deep fascia correspond to tendon cells and adapt themselves to the available scattered spaces between the collagenous bundles.

Macrophages[1] and "undifferentiated connective-tissue cells" are scattered through the deep fascia. The undifferentiated connective-tissue cells appear like the presumably fixed fibroblasts. During infection and wound repair many or perhaps all of the flattened fibroblasts become plump and active. It is believed that the macrophages play the part of scavengers removing cell debris from the connective-tissue spaces. The fibroblasts are the builders and produce collagenous fibers. *The term "undifferentiated connective-tissue cells" is a convenient one since it can be used whenever such phenomenon as new bone, fascia, tendon, or cartilage formation occurs.* It is possible, however, that the ordinary parenchymal connective-tissue cells in fascia and tendon can take part in new matrix formation as well as form collagenous fibers in wound healing. *In effect, the fibroblasts in many connective tissues may have the ability to revert to undifferentiated types under the influence of an unknown organizer.*

Since deep fascia is composed largely of dead intercellular substance rather than cells, the tissue requires little blood supply. It is therefore only sparsely supplied with blood vessels, and is a relatively non-vascular tissue. It contains both nerve elements and lymphatics.

Matrix

The manner in which fibroblasts lead to the deposition of collagenous fibers has been a source of controversy for years. It appears that the question has been settled by the animal experimental work of Wolbach in 1933 and that of Stearns in 1940. It was formerly believed that collagenous fibers were a transformation product of fibrin but Wolbach (5), in wound-healing experiments on animals, demonstrated that collagen first appeared as a diffuse deposit about the fibroblasts, which he interpreted as a secretion. He noted that the deposition of collagen cannot take place in animals deprived of vitamin C to the degree of absolute scorbutus. Collagen formation begins, however, 24 hours after the feeding of orange juice. Wolbach concluded that collagen is not

[1] Macrophages, unfortunately, are sometimes designated by various names such as histocytes, endothelial leukocytes, resting-wandering cells, etc.

and paratenon, which allow tendons to move freely during the contraction and relaxation of muscles.

Wherever a tendon lies in contact with another tendon or with a bony surface, the connective tissue may become so loose as to be converted into a circumscribed sac in which flattened fibroblasts, known as *mesothelium*, form a smooth slippery lining like the synovial membrane of a joint cavity. These sacs, called bursae, function as water cushions to lessen the effects of friction and pressure.

In addition to forming a general matrix throughout the body, connective tissue is also differentiated into rope-like bundles present in tendons, and well-defined sheets present in fascia lata, which form a tight-fitting sleeve for the thigh muscles.

In this chapter we are interested in tendon and deep fascia transplants and the two tissues will be considered together because of their similarity in structure and their almost identical behavior after free transplantation.

In general, *what is true for tendon grafts also holds for fascia grafts*, and fascia may be considered as a thin ribbon-like modification of tendon.

There is evidence that free transplants of tendon and fascia in contact with like host tissue resemble bone grafts in contact with bone. When the graft survives, as in autogenous cancellous bone transplants, the grafts heal by fibrous union with the host tendon or fascia, and the cells and graft structure survive as such. When tendon and fascia grafts fail to survive, however, the fate of the transplants is unknown.

DEEP FASCIA

The consistency of the deep fascia varies considerably in different parts of the body. Over the limbs, however, it is well defined as a tough sheet of white fibrous tissue which surrounds and supports the underlying muscles. In the palm of the hand and the sole of the foot it is unusually thick, forming a mechanically protective layer—the palmar and plantar fasciae (2). According to Clark the palmar and plantar fasciae represent the degenerated remains of the expanded tendons of muscles (the palmaris longus and plantaris), which in man have undergone considerable evolutionary atrophy.

Processes extend down from the deep fascia to form fascial septa which provide compartments for the separate muscle groups, bundles of nerves, blood vessels etc.

Some muscles take their origin from the deep fascia, and at these points the longitudinal collagenous fibers are interlaced with fibers running in other directions. Stripping the fascia with a fascial stripper at these points is difficult or impossible.

Clark (3) stresses an incidental function which may be ascribed to the ensheathing layer of deep fascia in the extremities. This is the promoting of circulation in venous channels and lymphatics in association with muscular activity. The limb musculature is contained within the deep fascia under some tension, as is shown by the bulging of muscle through an incision in the fascia. When the muscle contracts against the resistant fascial sheath, the compression of the soft walled veins and lymphatic vessels necessarily accelerates the flow of their contents in the direction determined by their valves. Veins and lymphatics outside the deep fascia are not subject to this mechanical influence, and in the lower limb they are, for this reason, particularly liable to varicosities and lymphedema in association with a poor circulation, developmental lymphatic insufficiencies, and other factors which are not known.

The tight sleeve-like quality of the deep fascia in the upper and lower extremities may be detrimental when infection or edema from pressure occurs within the deep fascial

Arrangement of Blood Supply

Tendon, like fascia, has few cells and a relatively large amount of collagenous intercellular material. Consequently it is rather sparsely supplied with blood vessels, since only a few are needed to provide substances for the cells. *The difference is in the arrangement of the blood vessel supply in fascia and tendon.*

Fascia is supplied by vessels which enter the tissue in numerous locations but in tendon blood vessels enter at both ends somewhat like nutrient vessels in bone. According to Clark (9), in the case of the long flexor tendons of the fingers, an additional vascular supply reaches them in the middle of their course by means of delicate reflections (or "mesenteries") of the mesothelial lining of the tendon sheaths, which are termed vincula vasculosa. When the loose and vascular paratenon[2] is removed from a tendon, one does not ordinarily observe any bleeding points on its white glistening surface, and free skin grafts do not "take" well over the exposed tendon. When the loose areolar tissue is removed from the outer surface of fascia lata, one notes a fair number of bleeding points, and skin grafts take well over the exposed deep fascia. Free skin grafts, however, will take well over tendon if it is covered by its thin vascular paratenon, or by a tendon sheath. The blood vessels supplying the tendon enter the tissue at both ends, from the muscle and also from the periosteum at the site where the tendon is attached to bone.

Nature undoubtedly refrained from providing tendons with a penetrating vascular supply at various strategic external points because of the long range of movement they possess. Vessels entering the substance of tendon at both ends provide a simple solution for a difficult circulatory problem.

The Gliding Mechanism

The loose areolar tissue on the surfaces of deep fascia provides a moderate amplitude of movement for its particular function. In some areas such as the patella and elbow, specialized bursae are present to permit movement with minimum friction. Tendons, however, have a wide amplitude of movement and it is therefore necessary to have a constant and well-developed mechanism for gliding. Bunnell (10), in his fine book, estimates the total amplitude of movement above the wrist as follows: wrist tendons, $1\frac{1}{4}$ inches; long flexors to fingers, $2\frac{3}{4}$ inches; long extensors to fingers and thumb, 2 inches.

According to Bunnell (11) the gliding mechanism of a tendon differs as to whether the tendon pulls straight or around a corner. In the former it travels through paratenon; in the latter, through a tendon sheath.

Paratenon is specialized loose tissue which fills in the space between the tendon and the immovable fascial compartment through which the tendon runs. Long elastic fibers in the paratenon are attached to the tendon and these are curled and tortuous like a spring when the tendon is at rest. When the tendon moves in either direction, these fibers straighten out long enough to allow free excursion. Thus the tendon does not actually glide through the paratenon but is adherent to it and merely drags the loose elastic tissue first in one direction and then in another.

In the tendon sheath formation, which is merely an adaptation for a tendon to turn a corner, the tendon does not move through any greater amplitude of motion than it does in the paratenon formation. It glides around a curve on a thin film of synovial fluid between two smooth synovial-lined (mesothelial) surfaces which serve as a water

[2] Mesotenon, paratenon, and peritenon are used variously by different authors to indicate the loose gliding connective tissue covering the outside of a tendon. In this book the term paratenon is used, as in Sterling Bunnell's work.

derived from fibrin in wound healing; collagen is secreted by fibroblasts, and collagenous fibers and reticular fibers are the same material in different physical states. Stearns (6) later, in 1940, demonstrated rather definitely that collagenous fibers are produced by the fibroblasts as a sort of budding-off process. The collagen material, after it becomes detached from the cell, differentiates into collagenous fibers which arrange themselves in directions according to the forces of stress and strain. Ham (7) states that mesenchymal cells develop into fibroblasts and then form abundant quantities of collagen and, in some instances, some elastin.

There is no direct evidence, however, that the fibroblast which produces collagenous fibers *can also elaborate elastic fibers*. Certainly elastic fibers are not produced in wound healing. New elastic fibers do not appear to be manufactured by any agency after a certain stage in the development of mammals. Elastin is a durable substance and nature apparently expects it to last for the life span of the mammal since no provision exists for its replacement. By special staining the author has demonstrated the presence of elastic fibers in the dermis of an autogenous human skin graft 40 years after free transplantation. Thus, elastic fibers may survive free transplantation and possibly remain during the life span of the human recipient. Clark (8) states that while the deposition of collagenous fibers is one of the main functions of fibroblasts, the developmental origin of elastic fibers is still obscure. The fact that their chemical and physical properties contrast so strongly with those of collagenous fibers suggests that they are derived from a correspondingly different source.

The individual collagenous fibers do not anastomose with each other. They are arranged in bundles and run a characteristically undulating course. Collagen being an albuminoid, the substance of these fibers on boiling becomes gelatin. Electron microscopic examination of collagenous fibers has revealed that the individual fibers are composed of fibrils with a segmented or striated appearance.

Elastic fibers, in contrast to collagenous fibers, run singly, branch frequently, and anastomose with one another. They consist of a very durable albuminoid called elastin, which is resistant to both acids and boiling. As seen under the electron microscope, they still appear as homogeneous structures which are not composed of smaller fibrils like collagenous fibers. Elastic fibers are sparsely present in deep fascia.

Collagenous fibers in deep fascia generally run a wavy longitudinal course; but in some areas where tensile strength is required in several directions, bundles of fibers are arranged in a number of planes and interwoven to form a sort of feltwork. Where this is present, removal of fascia with a stripper is very difficult.

The amorphous portion of the intercellular material consists of the usual hyaluronic acid and cement substances which surround the collagenous and elastic fibers and the cells. The whole tissue is pervaded and surrounded by tissue fluid, and substances pass from capillaries to cells and vice versa through this aqueous medium.

TENDON

The structure of tendon is so similar to that of fascia that it may be considered a thick rope-like form of fascia. It differs from fascia in its blood supply, the constant presence of a slippery channel to provide for movement, and more regular arrangement of the fibroblasts in long columns between dense parallel bundles of collagenous fibers. We can therefore safely state that the two tissues are alike structurally excepting for these three differences, which are described below.

CHAPTER 22

Transplantation of Fascia in Animals

Curiously the seventeenth and eighteenth century investigators were not concerned with fascia transplantation as they were with bone or cartilage, or even tendon. Interest in fascia grafts seems to have been delayed until early in this century, which has so predominantly been marked by the spirit of scientific research. This delay in concern with the transplantable qualities of fascia, and histological observations regarding the behavior of fascia grafts, may be due to the slow growth of knowledge concerning the anatomical and physiological properties of the tissue. It is more probable, however, that investigators did not become interested until a need for the clinical use of fascia grafts in the developing field of surgery became evident.

AUTOGENOUS, HOMOGENOUS AND HETEROGENOUS FASCIA GRAFTS

In 1908 Bogoljubow (1) reported on the use of aponeurotic strips resected from the rectus muscle of the dog and knotted over the pylorus in the same dog to obtain occlusion. Postoperative observations were made at intervals of 10 to 50 days. The transplanted strips showed no tendency to cut through the intestinal wall and separate into the lumen. There was definite narrowing of the intestinal lumen at the site of the aponeurotic strips, and the abdominal wall showed no necrosis.

In the first systematic experimental study of transplantation of free grafts of fascia, Kirschner (2) in 1909 removed pieces of fascia lata from rabbits and replanted them in the defect. The sides of the autograft were sutured to the structure surrounding the defect. The fascia remained alive, showing no necrotic areas. Only in the course of weeks and months did the fascia lose its inner characteristic structure and assume the appearance of a firm tendon-like connective tissue. In free fascia transplantation under functional stress a defect in the Achilles tendon of the dog was bridged with fascia; this likewise remained alive. Kirschner concluded that fascia at least may be used as a tendon substitute. He noted that it offers an almost unlimited material for transplantation.

Von Saar (3) (1910) was the first to consider the permanent viability of fascia transplants as questionable. Experimentally he replaced fascia in dural defects and perceived evidences of degeneration in the course of two weeks.

In the animal experiments carried out by Lewis and Davis (4) (1911) [autogenous] pieces of fascia lata or the sheath of the rectus abdominis were dissected loose and either buried under the subcutaneous fat of the abdomen or replaced free in the fascia area from where they had been separated (under the skin of the thigh) in dogs. Obser-

cushion. The inner layer of the tendon sheath which intimately invests the tendon is called epitenon, and the outer layer is called the sheath. Small septa running into the tendon from the epitenon are called endotenon, which is similar to the endomysium of muscle. Bunnell (12), in agreement with Clark (9), states that blood and lymph vessels from the tendon sheath nourish the tendon itself. In the paratenon formation this does not appear to be true.

Tendon which has been denuded of its sheath or its paratenon, as previously stated, is not a good host tissue for free skin grafts. When a tendon has become exposed, it is wise to make provisions for a new gliding mechanism to replace the absent sheath or paratenon.

Tendon Cells

The characteristic tendon cells in tendon are the parenchymal cells of the tissue, since they are the only fixed cells present excepting for fibroblasts in the stroma which separate the tendon bundles. They differ from the flattened, scattered fascial fibroblasts by their unique arrangement in long parallel columns in spaces between the thick parallel collagenous bundles.

The cells in their parallel columns are rod-shaped when seen in profile. The cytoplasm takes a dark stain with basic dyes and the cells contain a single nucleus. Although the limits between the successive cells in a row are distinct, the lateral limits of the cells are indistinct, because here the cytoplasm continues in a thin membrane. According to Maximow and Bloom (13), this cytoplasmic extension can sometimes be followed to another cell row.

The inactive tendon and fascial cells have low metabolic requirements while simply playing their part in servicing and maintaining their intercellular materials. When injury occurs, however, both are capable of becoming active in the production of new collagenous fibers for the process of repair, according to some authorities. Others hold that healing occurs through the activity of infiltrating fibroblasts from the connective tissue outside the tendon and fascia.

The fibroblast is a specialized and a nonspecialized cell. As the parenchymal cell in fascia and tendon it can both maintain and service its specific tissue and possibly also repair its tissue when the need arises. The fibroblast as a specialized cartilage, bone or fat cell in adults, however, can only service and maintain its tissue; it probably does not produce new cells of cartilage, bone, or fat after injury in humans. The parenchymal cells in adult human cartilage and in bone also appear to lack the ability *to produce new intercellular matrix.*

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After removing fascia lata from the thigh in dogs, Valentin (10) (1913) either immediately transplanted it into the cavity of the peritoneum of another dog with silk button-sutures, or first immersed it in physiological salt solution at body temperature until the implantation site was prepared. After a few days to 296 days there was evidence of successful homoplastic transplantation of fascia lata in the abdominal cavity, and the fascia healed in largely with its own structure and with characteristic nuclei, without being replaced by connective tissue. Only at the ends where constriction occurred due to the silk thread did isolated bundles disintegrate.

Kolb (11) (1913) laid autogenous fascial strips from the abdominal fascia, freed of muscle fibers, around loops of the large and small intestine and around the duodenum in rabbits and noted secondary shrinkage of free fascia grafts.

Kleinschmidt (12) (1914) stretched autogenous fascia lengthwise and transversely in complete defects of the M. quadriceps, and transplanted autogenous fascia under the skin, without functional stress, in rabbits. He noted that fascia remains alive, as evidenced by vital staining. Kleinschmidt recommended the use of fascia in its lengthwise direction for bridging defects in organs of motion, because the long fibers resist the adjoining tissues for the longest period. The most numerous and strongest elastic fibers run in the same direction as the collagenous fibers.

Von Eberts (13) of McGill University transplanted a segment of autogenous fascia lata from the iliotibial band to a defect produced in either the Achilles tendon, the dura mater, or the peritoneum. Microscopically the fascial elements appeared unaltered. The inflammatory changes were associated with the adhesions formed between the graft and the host tissue and were characterized by exudation of lymph and proliferation of connective-tissue cells derived from the inner surface of the tendon sheath. There were no signs of vascular invasion of the graft or of multiplication of fascia cells. There was no necrosis present.

Greggio (14) (1914) completely resected the muscular layer at the level of the superior part of the abdomen in dogs. In some instances the peritoneum remained intact. A strip of autogenous femoral aponeurosis was transplanted to replace the loss of substance in the abdominal wall, being sutured to the superficial aponeurosis of muscle. The dogs were sacrificed from fifty to 200 days postoperatively. Microscopically, the aponeurotic fibers and fibrils, with their special characteristics, persist in the new site even in conditions only slightly favorable to their vitality. With cicatricial tissue which surrounds them and can penetrate between them, they form a very resistant whole.

After performing gastrojejunostomy on the dog Gibson and Beekmann (15) (1915) carried out pyloric occlusion with autogenous fascial bands obtained from fascia lata or over the rectus sheath. This resulted in complete functional occlusion in one instance. In the instance of incomplete occlusion after eighty-eight days postoperatively the pylorus was covered with adhesions, the fascia transplant present causing stenosis. Their experimental results obtained by fascia transplantations were more or less borne out by clinical applications. Gibson and Beekmann believed at that time that application of a free flap of fascia, when it can be applied, promises the best result. No histological examinations were made.

Experimenting on dogs Neuhof (16) (1916) either firmly ligated the pylorus with a strip of fascia lata knotted about it, or snugly sutured a broad fascial band around the pylorus and enfolded it by two or three tiers of sutures. Only very temporary pyloric exclusion resulted from ligation with fascial bands. Crushing the pylorus or application of irritants to the pyloric muscoa had no

vations were made at intervals of 4 to 35 days. In their opinion, the changes in form and size of the transplanted fascia depended on the direction of the planes of the fibers. Fascia pieces when not sutured in position curled up. In ten days the transplant was adherent to the fascia lata and preserved all the characteristics and microscopic features of normal fascia. Histologically, the fibers reacted normally to stains. After 23 days the transversely-cut fibers stained normally; leukocytes and round cells infiltrated the margins, with little invasion of the transplanted tissue. After 35 days the transplant was firmly adherent to the subcutaneous fat and was considerably reduced in size but it was viable and still retained all the physical characteristics of fascia.

In numerous experiments on dogs, Davis (5) (1911) transplanted fascia lata from the thigh, or strong abdominal fascia, in single and double layers, or twisted, as autografts and homografts. The fascia was kept in the refrigerator for as long as 7 days, in cold storage in gauze moistened in salt solution or in normal salt solution for 56 days. Fascia kept in normal salt solution in cold storage showed edema in the superficial connective tissue when first removed, whereas fascia kept in moist salt gauze in cold storage was normal in appearance. It seemed to Davis that suitable fascia might be preserved until required for clinical use. In every instance the fascia retained its own structure and seemed to be well nourished. After removal from its recipient bed it was found to be as tough and strong as when first transplanted. There was no untoward effect in use of the limb after removal of fascia lata.

In animal experiments by Rittenhaus (6) (1911) a defect, produced by removal of a flap of the abdominal wall consisting of all the layers except the peritoneum, was closed partly with fascia lata from the thigh, and partly by a fascial flap from the removed part of the M. oblique externus. The wound healed by primary intention except for the formation of a suture fistula. When observation was made at 4½ months postoperatively, the abdominal wall at the site of the defect was as resistant as the other parts. The fascia remained viable in its whole extent and covered the defect with dark connective-tissue membrane.

In experiments on dogs Levit (7) (1912) covered defects in the abdominal wall with fascia lata, replacing muscle, fascia, and peritoneum, with ideal healing and good clinical results.

Halstead gave credit to Francesco Nassetti of the University of Siena for the idea of constricting blood vessels with bands of fresh tissue in 1912. Experimentally, a few months later Halstead (8) (1913) partially occluded the aorta in the dog by winding homogenous spiral strips and cuffs of fresh aorta, and also fascial bands around it. There was no diminution in the femoral pressure, and the band was found to have relaxed and to have been partially absorbed. After 7 months there was considerable absorption of the band and in one instance only a trace of it remained.

In rabbits and dogs Valentin (9) (1912) used an autogenous fascial flap taken from the thigh to cover a defect produced in the peritoneum. The fascia was observed to remain viable for the most part. He also substituted homogenous pieces of fascia lata for a piece of peritoneum in animals. The homografts of fascia grossly showed no peculiarities; they were well defined against the peritoneum. The fascia showed good staining and a normal appearance. Microscopically, there was an extraordinary, strong reaction in the sense of infiltration of the tissue with leukocytes and the rich formation of young connective tissue. The transplanted fascia was enclosed in a fibrin mass, which was organized and already partly penetrated by young connective tissue between the bundles of fascia.

which were vascularized by the subcutaneous and subperiosteal connective tissues. There were no signs of resorption or degeneration. Greggio believed that his experiments demonstrated the utility of aponeurotic grafts in general surgery.

Neuhof (19) (1918) pointed to the great advantage of auto- over homo- and hetero-transplantation of tissues for replacement of total defects of arteries as definitely established. In experiments on dogs with autogenous fascia transplants some were followed for six to nine months. A section of the thoracic aorta was removed and into the arterial defect was placed fascia lata taken from near the knee joint and made into the desired shape and of a slightly larger size than the section removed, with its smoother surface facing the lumen. The edge of the fascia and the cut edge of the artery were approximated with suture.

Seven months after the fixation the site of the transplantation was a smooth, glistening surface. The pulsations of the segment containing the transplant and of the adjoining segments were normal. The size of the fascial patch was the same as when transplanted. There was smooth continuity with the adjoining aortic lining below, and abrupt demarcation between the transplant and the vessel intima above. The site of the defect was occupied by very closely-woven connective tissue, rather richly supplied with nuclei toward the free surface, and less and less toward the depth. The free or lumen surface was covered by flat endothelial-like cells. In experiments on the abdominal aorta, fusion between the tissues occupying the defect and the adjoining aortic tissues was very intimate, so that the line of transition could not be recognized.

Neuhof believed that the serviceable replacement of defects of major arteries with fascia thus demonstrated offered great possibilities for clinical application in human surgery.

In the experimental work of Gallie and Le Mesurier (1921) autogenous patches of fascia were excised from rabbits, immediately replanted in the site and sutured. During the first few weeks an inflammatory reaction occurred in the surrounding tissues and a vascular areolar membrane was formed. The transplant remained alive and healed to the surrounding tissues by means of newly-formed connective tissue. Muscle aponeurotic transplants behaved exactly as did the pieces of deep fascia. There was no evidence of proliferation of the essential cells of the tissue. The connective tissue which formed from host tissue at the point of union produced a scar. Weeks or months after operation the sutures had cut out or were absorbed, and the cut edges of the fascia or aponeurosis had separated widely owing to the stretching of the scar or areolar tissue which constituted the line of union (20).

In still another series of experiments, an autogenous piece of fascia removed from the rabbit's back was placed transversely across the middle of the gap and woven two or three times into its edges. The edges of the fascia were held apart by the transplant. The strength of the union of the transplanted fascia to the edges of the gap depended on the extent to which the fascia was woven into the transplant. The strength of union also depended on the care with which the portion of the fascia was cleared of areolar tissue. Union occurred by newly-formed connective tissue. To make this union strong enough to stand normal physiological strain, long strips of fascia from the rabbit's back threaded into a needle were passed through the edge of the fascial gap and then through that of the opposite side, *thus serving as a living suture* (20).

Specimens recovered at intervals of a week up to a little more than a year showed that the fascial sutures behave in practically the same way as the transplanted fascia. Fibers and cells remained unchanged. After a year the sutures appeared as a white rounded

helpful effect. He considered that disturbed mechanical arrangement following incomplete occlusion may be fatal. He did not think the method merited clinical application on the basis of the experimental results.

In a series of animal experiments in 1917 Neuhof (17) implanted fascia into visceral defects. He considered the details of technique responsible for obtaining satisfactory results in some of these experiments. Autogenous fascia from the abdominal wall was used in a small number of animals; fascia lata, in all later ones. A sheet of fascia lata was sutured in a defect produced in the vertex of the bladder in dogs. Upon removal it showed hyaline degeneration. Layers of the bladder wall were united to the transplant by a very thick layer of film. Fascial replacement of vesical defects was considered adequate. Fusion between the tissues of the defect and those of the bladder wall was complete. Neuhof noted bone formation in the defect and smooth muscle across it.

Granulation tissue appeared on the surface of fascia implants in urethral defects soon after operation. In the later stages the defect was solidly filled with connective tissue, partly contracted, and entirely covered by epithelial overgrowth.

An autogenous sheet of fascia from the abdominal wall was implanted near the pylorus. In all instances the transplanted fascia was attacked by the gastric juice and perforation occurred. When gastroenterostomy, with and without pyloric exclusion, was added to fascia replacement of the pyloric defects, healing occurred.

Fascia transplants in defects in the upper part of the small intestine generally failed; small transplants in the lower part were often successful. But replacement of larger defects not infrequently failed. Fascia implantation in large defects in the large intestine succeeded in two out of three experiments. Neuhof held such fascial repair of intestinal defect as an unreliable procedure.

In implantation of fascia in tracheal defects the ends of the cartilage rings showed active proliferation. Fusion between the fascia and the tracheal tissues, and overgrowth of ciliated tracheal epithelium were complete. The characteristic appearance of the transplant was still moderately well preserved 8 weeks postoperatively.

In fascia implantations in pleural defect, 5 months after operation the thoracic wall showed firm adhesions between the lung and transplant. The transplanted fascia loses its characteristic structure relatively soon after operation.

In the limited time of observation of fascia transplanted in defects in the dog's lung, there was no evidence of an extension of pulmonary pleura over the transplant.

Implantation of fascia in pericardial defect was not entirely satisfactory due to fatal pleural complications.

Fascia placed in defects of the diaphragm provided lasting barrier to hernia, union between the fascia and muscle of the diaphragm taking place. Regeneration of the muscle across the defect did not occur.

In the case of implants in defect of the liver, the grafting after 6½ months remained intimately adherent to the original raw area, a very close fusion having occurred. Well-stained liver cells were found in the substance of the altered fascia.

There was no leaking of cerebrospinal fluid in fascia implantation in defects of the spinal dura. In two months the wound had healed firmly, muscle being adherent to the surface of the transplant. The characteristic structure of the transplanted fascia had largely disappeared, the defect being occupied by connective tissue (17).

In another experimental research Greggio (18) transplanted autogenous pieces of femoral aponeurosis into a wound produced by complete resection of a muscular surface in the abdominal wall. Histological examinations made from 350 to 750 days after operation showed the perfect vitality of the grafts,

veloped vascular connective-tissue mantle is formed; this envelops the fascia through nutritive, reparative and appositional activity. In the composition of the fascial end-product in homoplasties there occurs without a doubt a wider space for the appositional processes coming from the stroma than in fascial autoplasties. Rehn believed that autogenous fascia grafts survived as such to a large extent and were therefore preferable to homografts.

Becoming aware of the successful use of dead tendon grafts by French investigators, particularly Nageotte, Koontz (25) (1926, 1927) conceived the idea that dead fascia grafts were also a possibility. Pieces of fascia from fascia lata and the sheath of the rectus were preserved in 70 per cent alcohol for varying periods of time (3 to 75 days), and were transplanted into defects produced in the sheath of the rectus or fascia lata in cats and dogs. Some of the grafts were autografts; others, homografts and still others were from different species. The animals were sacrificed from 2 to 7 months postoperatively. Microscopic sections showed that in nearly all, living fibers had become interwoven with those of dead tissue, without evidence of absorption. Living cells seemed to have wandered among dead fibers, so that "the former dead graft is now living tissue."

In addition to fascia lata and the sheath of the rectus of dogs and cats as grafts, among the materials preserved and transplanted were fascia lata, the pericardial sac of the ox, and the submucous coat of pig's bladder. Histologically, the dead cells of the graft had been replaced by living cells from the host, the graft had become firmly fixed to the surrounding tissues by ingrowth of fibroblasts, and the implanted tissue had become vascularized. Several of these materials were sutured over ventral hernias produced in dogs, ox tissue giving the best results. Except for the presence of the sutures it was impossible to determine the defined limits of the dead graft.

In a study by McNealy and Lichtenstein (26) (1928) autogenous fascial strips taken from the anterior layer of the rectus sheath in dogs were freed of fat, split longitudinally and then placed under a vessel. One end was threaded through a slit at the opposite end, thus forming a fixed loop about the vessels. The fascia was drawn through the muscle fibers. One end of the fascia was fixed by tension, the other end was put under gentle traction. At intervals of two weeks to four months observations were made. There was complete occlusion of the femoral artery at the site of the fascial strip. No apparent destruction of the intima was evident. The fascia transplantation remained fixed; firm fibrous union occurred between the connective tissue of the muscle bundles and the fibers of the fascial strips. Such strips are non-irritating to the surrounding tissue.

Rosenblatt and Meyers (27) (1928) resected a portion of the sheath of the rectus muscle in dogs and sutured the edge of the muscle under tension to the reflected edge of the external oblique by single sutures of ox fascia or ox tendon. These heterogenous suture materials were first preserved in 70 per cent alcohol, the areolar tissue removed, and the tissue cut into strips, which were again placed in alcohol. All animals in which dead fascia was used as suture showed firm union of muscle to fascia; autogenous fascia resulted in no firmer union. Preserved ox tendon was not nearly so satisfactory in result or in handling as ox fascia. It appeared to Rosenblatt and Meyers that the fascia graft acted as an element for substitution of connective tissue and was in part absorbed. There was some foreign-body reaction.

Experimenting with dogs Koontz (28) (1929) used alcohol-preserved ox fascia successfully to repair defects in the stomach wall, in the bladder, and to reinforce the

cord, closely resembling tendon. They consisted of parallel fibers, with a few vascular areolar tissue trabeculae (21). Gallie and Le Mesurier held that these experiments demonstrated the clinical value of the principle of the use of living sutures.

In a very comprehensive article, Gallie and Le Mesurier (22) (1924) described their experimental findings regarding the behavior of autogenous fascial transplants in animals which had been buried for long periods of time. In animals (rabbits) patches of fascia, tendon, and aponeurosis were excised and immediately sutured back in their original positions. The specimens were recovered at intervals ranging from a few days to many weeks. During the few weeks after the usual inflammatory reaction a thin transparent film developed over the transplant consisting of capillaries and fibroblasts. This film increased in thickness and by the end of the third week had developed into a mass of spindle-shaped fibroblasts and collagenous fibers which formed a capsule about the graft. During this time the transplant remained alive and, on microscopic examination, showed little change beyond a moderate edema. There was no evidence of invasion by new blood vessels and no leukocytic infiltration.[1]

After the third week the inflammatory reaction in the host tissue subsided and in specimens recovered as late as a year after the operation there was nothing to indicate that the cells or fibers had been changed in any way. The fascia and tendon cells appeared like living cells in both the early and late sections excepting that thick tendon grafts showed a central necrosis. Gallie and Le Mesurier stressed the importance of broad attachment of fascia and tendon grafts for firm union, and the value of fascia grafts as "living sutures." Autogenous fascial sutures buried up to two years appeared like normal fascia both grossly and microscopically. On the basis of this animal experimental work they successfully used fascia and tendon clinically for a wide variety of purposes. Thus the term "living suture" has become associated with the names of two individuals who thought in terms of living tissue cells.[2]

Reid (23) (1924) reported experiments on dogs in which the lumen of the aorta was reduced to about a third of its normal size by suturing, so that it was temporarily occluded above and below the point of constriction. Observations extended over a period of two to eight months. In each instance partial occlusion persisted. A dense fibrous connective-tissue reaction occurred about these mattress sutures. A small strip of fascia taken from the anterior sheath of rectus muscle was made into a plug and introduced into a slit in a segment of aorta. The vessel wall below and at the site of the occluding fascial plug underwent marked atrophy.

Rehn (24), who had worked with homoplastic fascia transplant experimentally, considered in 1924 that it had little practical meaning, since sufficient autoplastic material is available. He had proved the histological retention of the fascia homograft and established that after the lapse of a half year it had changed into living tissue in the host. In summary, first a very richly-de-

[1] In the author's experience with humans, blood circulation in autogenous fascia grafts was established in transplants buried for about three days, often associated with numerous areas of cellular exudate within the graft structure. In three weeks the exudate, which included leukocytes (polymorphonuclears), had disappeared but the engorged vessels were still present and continued to remain in the graft structure up to about four months after transfer. The fibroblast cells in the grafts appeared viable, had dark staining nuclei and seemed to be more numerous than those seen in control sections of fascia in grafts buried for 1½ years. The endothelial cells lining blood vessels in fascia also survived as such.

[2] This article should be read in the original by all who are interested in tendon and fascia transplants.

action in the surrounding tissue than did live fascia. Haas recommended the use of live fascia in union with muscle clinically if it is possible, because the autogenous living fascia appears to be transformed into tendinous tissue more rapidly.

Tschmarke (31) (1933) resected fascial pieces from the thigh, fixed pieces loosely, bridged fascia, implanted rolled fascial strips, and placed strips of different widths around the arteria femoralis in dogs as autografts. He concluded that free transplanted fascia possesses a general tendency to secondary shrinkage. This tendency was traced back to processes which are conditioned by injury to the transplanted tissue, a parallelism existing between the degree of tissue injury, and the intensity and extent of the shrinking process. The shrinking intensity of injured transplants was proved by narrowing large arterial channels in experiments on dogs.

In experiments on dogs, Chouké and Whitehead (32) (1941) sutured autogenous fascia to fascia, and in some, muscle to fascia. The fascia was exposed and freed of superficial areolar tissue, and sutures were placed at right angles to the incisions. The animals were sacrificed at periods varying from 8 to 142 days, a large piece of fascia containing the operated tissue being removed, and examined microscopically. When closely approximated, fascia united readily to fascia by connective-tissue growth. The type of suture material did not appear to be concerned in the union.

Foshee (33) of Grand Rapids made a thorough investigation of the formation of regenerated fascia at the thigh donor sites in dogs. A piece of fascia lata removed from the dog's thigh when microscopically examined showed it contained three layers identical to that of man: i.e., an inner and outer transverse layer, and a middle longitudinal layer. "The inner and outer transverse layers regenerate by extension directly from their respective layers of the adjacent normal fascia lata together with their sheaths and blood vessels. The middle longitudinal layer does not regenerate." At the end of 2 weeks the regenerated fascia lata at the donor site in a medium-sized defect is only a thin veil. At one month it is half as thick as normal fascia lata; at two months it is grossly and microscopically as thick as normal fascia lata. After four months two layers of superficial fascia, both hypertrophic, were found in the dog. A very large defect may show a slower process of regeneration; a smaller one may show such rapid regeneration that in one month the fascia lata will have the thickness of normal fascia. In the absence of the middle layer the inner and outer layers undergo hypertrophy and hyperplasia in compensation

Gallie (34) (1948) expressed the belief that fascia lata may be transplanted from one place in the body to another and continue to live, as demonstrated by histological studies and clinical experience. In a summary of the histological changes he stated that mild inflammation, without any marked change except moderate edema, occurs in the graft itself. The cells and fibers continue to stain well, thus showing no necrosis. There is no evidence of invasion by capillaries or infiltration with leukocytes. In specimens recovered years after transplantation there is no indication of any great changes in the tissue, or of marked disturbance in its physiological value.

In experiments on dogs Weckesser *et al.* (35) (1949) used the outer slippery layer overlying autogenous fascia lata, human fibrin film, bovine fibrin film, gelfoam, cellophane, and oxycell cotton to wrap completely around a tendon prior to wound closure. The flexor carpi ulnaris tendon of each leg was exposed and (the tendon) traumatized. In one instance human fibrin film was placed around one tendon and under the other. The smooth slippery surface of the

suture line at the point of anastomosis in the small intestine. Digestion of the graft in the stomach was prevented by giving bismuth subnitrate orally. The fascia graft in the stomach and bladder became covered with fibrin, which was transformed into connective tissue. In 8 months the fascia graft in the stomach wall was pervaded by a fine network of blood vessels. In the bladder the connective tissue became covered with glistening serosa, to which were attached a few omental adhesions. Microscopically sections showed the mucosa over the graft to be lined with the usual stratified epithelium of bladder mucosa. In the intestine the fascial cuff became organized with the serous coat of the intestine and was also covered with a glistening coat. There were very few adhesions.

Horsley (29) (1931) compared the reaction of autogenous fascia transplant and that of alcohol-preserved fascia lata of the ox when tied loosely about the small bowel in the peritoneal cavity, snugly about the pylorus, and placed in the abdominal cavity of the dog for 5 days to slightly more than 6 months. In these sites the dead preserved fascia of the ox and living autogenous fascia react similarly and without encapsulation. Both types of fascia atrophy and stretch when placed in the peritoneal cavity but not in the abdominal wall. The living fascia seemed to occlude the pylorus several weeks longer than the dead ox fascia. If infection was present, both living and dead preserved ox fascia were broken down and absorbed. Curiously, the alcohol-preserved fascia of the ox did not react in humans as it did in the dog. It was quickly absorbed and lost its tensile qualities, while autogenous fascia lata apparently retained its strength. This difference, as Horsley suggested, may be due to the fact that man is higher in the biological scale than the dog or ox; the tissues being more complex and the proteins of the fascia lata of the ox being more foreign to man's tissues than to the tissues of the dog. That one of man's chief articles of food is beef may be a factor, increasing the rapidity with which foreign beef proteins are absorbed in man.

Haas (30) (1930, 1931) carried out experiments on dogs in which a piece of live autogenous fascia lata was removed from the outer side of the thigh and sutured to the cut end of one of the hamstring muscles with silk. Six days postoperatively the transplanted fascia was found to be firmly united to the cut end of the muscle. The union became firmer with lapse of time, and at 80 days the transplanted fascia took on a tendinous structure. Microscopically it was observed that the endomysium and the perimysium of the muscle played a major role in forming union with the fascia. The muscle cells appeared to have assumed an active part in the uniting process. The muscle cells became transformed into elements of fibrous tissue which intermingled with the transplanted fascia.[3] There was also evidence that the connective-tissue fibers of transplanted fascia played an active part in the union.

Fascia lata, presumably autogenous, preserved in alcohol, was sutured in a defect produced by removal of tendon and muscle from the leg. Grossly and microscopically the dead preserved fascia lata was shown to have united just as rapidly and firmly as did live fascia lata. Union of both dead and living fascia depended mainly on the ingrowth of endomysium and perimysium. There was some evidence that preserved fascia lost some of its elasticity and stirred up more re-

[3] One wonders on what factual evidence Haas was able to determine that muscle cells assumed an active part in the healing process. Probably this was accomplished by the fibroblast cells in the endomysium and not by muscle cells. Certainly there is no evidence that the specialized multinucleated muscle cell can become a fibroblast and produce the associated collagenous fibers which constitute fibrous tissue.

action in the surrounding tissue than did live fascia. Haas recommended the use of live fascia in union with muscle clinically if it is possible, because the autogenous living fascia appears to be transformed into tendinous tissue more rapidly.

Tschmarke (31) (1933) resected fascial pieces from the thigh, fixed pieces loosely, bridged fascia, implanted rolled fascial strips, and placed strips of different widths around the arteria femoralis in dogs as autografts. He concluded that free transplanted fascia possesses a general tendency to secondary shrinkage. This tendency was traced back to processes which are conditioned by injury to the transplanted tissue, a parallelism existing between the degree of tissue injury, and the intensity and extent of the shrinking process. The shrinking intensity of injured transplants was proved by narrowing large arterial channels in experiments on dogs.

In experiments on dogs, Chouké and Whitehead (32) (1941) sutured autogenous fascia to fascia, and in some, muscle to fascia. The fascia was exposed and freed of superficial areolar tissue, and sutures were placed at right angles to the incisions. The animals were sacrificed at periods varying from 8 to 142 days, a large piece of fascia containing the operated tissue being removed, and examined microscopically. When closely approximated, fascia united readily to fascia by connective-tissue growth. The type of suture material did not appear to be concerned in the union.

Foshee (33) of Grand Rapids made a thorough investigation of the formation of regenerated fascia at the thigh donor sites in dogs. A piece of fascia lata removed from the dog's thigh when microscopically examined showed it contained three layers identical to that of man: i.e., an inner and outer transverse layer, and a middle longitudinal layer. "The inner and outer transverse layers regenerate by extension directly from their respective layers of the adjacent normal fascia lata together with their sheaths and blood vessels. The middle longitudinal layer does not regenerate." At the end of 2 weeks the regenerated fascia lata at the donor site in a medium-sized defect is only a thin veil. At one month it is half as thick as normal fascia lata; at two months it is grossly and microscopically as thick as normal fascia lata. After four months two layers of superficial fascia, both hypertrophic, were found in the dog. A very large defect may show a slower process of regeneration; a smaller one may show such rapid regeneration that in one month the fascia lata will have the thickness of normal fascia. In the absence of the middle layer the inner and outer layers undergo hypertrophy and hyperplasia in compensation

Gallie (34) (1948) expressed the belief that fascia lata may be transplanted from one place in the body to another and continue to live, as demonstrated by histological studies and clinical experience. In a summary of the histological changes he stated that mild inflammation, without any marked change except moderate edema, occurs in the graft itself. The cells and fibers continue to stain well, thus showing no necrosis. There is no evidence of invasion by capillaries or infiltration with leukocytes. In specimens recovered years after transplantation there is no indication of any great changes in the tissue, or of marked disturbance in its physiological value.

In experiments on dogs Weckesser *et al.* (35) (1949) used the outer slippery layer overlying autogenous fascia lata, human fibrin film, bovine fibrin film, gelfoam, cellophane, and oxycell cotton to wrap completely around a tendon prior to wound closure. The flexor carpi ulnaris tendon of each leg was exposed and (the tendon) traumatized. In one instance human fibrin film was placed around one tendon and under the other. The smooth slippery surface of the

fascial layer was placed next to the tendon. Human and bovine fibrin film were still present, usually thinned out, and cellophane remained apparently unchanged at the end of four weeks. Fibrin film of bovine or human origin as well as cellophane produced a zone of vascular connective tissue about the tendon. Human fibrin film, bovine fibrin film, and cellophane were most effective in preventing adhesions about injured tendons. Autogenous fascia was of definite value but less than the others. Weckesser and his colleagues, however, concluded that film materials may seriously interfere with the nourishment of the tendons.

Cuboid masses of muscle with attached fascia were resected from the back in rabbits by Andresen, Monroe and Hass (36) (1952). Autogenous transplant of fascia was transferred to the defect on one side and an homogenous muscle transplant, to the other side of the recipient. Two simultaneous autogenous transplants and two homogenous transplants were transferred to paired animals of the same strain, which were sacrificed at varying periods of weeks. Sections were removed in the direction perpendicular to muscle fascia.

The basic pattern of degeneration and granulation tissue organization of both autogenous and homogenous grafts was essentially identical. Lymphocytic infiltration and angeitis were located in the musculofascial zone of the transplant, from where they spread through fascia into the overlying pannus of granulation tissue. The lymphocytic and mild angeitic forms of reaction predominated in the case of single homotransplants, becoming conspicuous at the end of two weeks. When multiple successive homotransplants were made from the same donor to the same recipient, acute angeitis with thrombosis occurred, and the lymphocytic reaction failed to develop or persist. Multiple successive autotransplants, on the other hand, did not influence the type or degree of reaction to autotransplant in the same animal. There was no evidence that autotransplants had any influence upon the sequence of reactions to homotransplants, or that the presence of homotransplants influenced the nature of the reaction to autotransplants in the same animal.

SUMMARY COMMENT ON FASCIA GRAFTS IN ANIMALS

Autogenous Fascia Grafts

Kirschner in 1909 reported the successful use of autogenous fascia grafts and stated that the transplants retained their structure after transfer and remained in the host area as living tissue. Von Saar in 1910 questioned the permanent viability of fascia transplants. The conflicting opinions of these two investigators have been confirmed or repudiated by all later workers with fascia transplants. Very little that is new has been added to our knowledge of autogenous fascia transplants in animals by later investigators.

It is interesting to note that whenever an investigator finds that a buried tissue graft survives as such, later investigators inevitably fill the vacuum of opposite opinion and maintain that the cells in the graft are replaced by host tissues. In regard to much more complex surface tissue grafts such as skin and mucous membrane there is no conflicting opinion, since the course of these grafts can be readily followed, and all believe that skin and mucous membrane grafts do survive as such. At any rate the epithelial layers survive, and the glands and hairs in the dermis of the skin survive. In the case of the dermis it could be argued that infiltrating host fibroblasts gradually replace fibroblasts in the transplanted dermis but there is no positive evidence that this does occur.

Kirschner's report regarding the survival of autogenous fascia grafts in animals stimulated a great interest in their clinical use, and many experiments were undertaken on

animals to determine the variety of clinical application in man.

John Staige Davis, Kleinschmidt, and Greggio, on the basis of animal experiments, concluded that autogenous fascia grafts survive as such after transplantation, with living fascial cells, and normal intercellular structure. Von Saar's opinion that autogenous fascia grafts did not survive after transfer was supported by Neuhof. The latter, however, believed that the grafts were useful clinically, since infiltrating host fibroblasts gradually replaced the cells in the fascia transplant, serving as substitutes for the original cells. Specific mention is not made regarding the fate of the collagenous fibers and elastic fibers in fascia grafts, and no investigator comments on the survival or replacement of blood vessels in the grafts.

Gallie and Le Mesurier, and Rehn, who are all known as careful workers, demonstrated that autogenous fascia transplants remain alive after transfer and heal in firmly with the surrounding host tissue, and the work of these men is impressive. Later Gallie advocated the use of autogenous fascial strips as "living sutures" in hernial repairs. His later experimental work confirmed earlier observations that the cells in the graft survive and retain their normal collagenous matrix.

It appears that the preponderance of opinion favors the viewpoint that free autogenous fascia grafts in animals remain viable after transfer. The belief that the cells in fascia grafts are gradually replaced by infiltrating host cells, however, still continues to have supporters, but it is based on negative rather than positive evidence. These later investigators also do not make any definite statement concerning the survival of the blood vessels in the grafts, with their lining endothelial cells, or the retention of the collagenous fibers and sparse elastic fibers in the matrix of the graft. Presumably the new infiltrating host fibroblasts replace the original fibroblasts but accept the intercellular substance, which they service and maintain. There are no definite statements regarding the production of new collagenous fibers by the host fibroblasts which are assumed to have taken over control of the graft structure.

Homogenous and Heterogenous Fascia Grafts

Although Valentin concluded that the cells in fresh homogenous fascia grafts survived after transfer, Rehn and others preferred the use of autogenous grafts and believed that fresh homografts were changed into host tissue and that the original cells were replaced by host fibroblasts. The majority of recent investigators support the viewpoint that the fibroblast parenchymal cells in fresh homografts die and are replaced by host cells. There are no definite conclusions regarding the behavior of endothelial cells lining the blood vessels in fresh homogenous fascia grafts, but presumably these cells also fail to survive. Evidence has been presented that the collagenous fibers in fresh fascia homografts are slowly absorbed and partly replaced by host fibroblasts.

The cells in heat-treated and preserved homogenous fascia grafts (other than frozen grafts) are dead at the time of transfer. These dead cells are also replaced by infiltrating host fibroblasts, but the fate of collagenous fibers in the graft is not definitely known. No investigator has commented on the fate of the sparse but durable elastic fibers in homogenous fascia grafts, either fresh or preserved.

Rosenblatt and Meyers concluded that preserved ox fascia grafts were of as much value as autogenous fascia grafts, since the former were replaced by host tissue as fascia. Others preferred the use of autogenous fascia grafts, believing that preserved heterogenous fascia grafts gave rise to more cellular reaction in the host tissues. The eventual fate of heterogenous fascia grafts in animals is

not definitely known at this time. The factual evidence from microscopic examination of the heterografts indicates that the foreign graft is replaced by host tissue. Some state that the host replacement tissue is a duplicate of the original graft, but others believe that the graft structure is replaced by ordinary scar tissue, which does not have the tensile strength or structure of true fascia.

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CHAPTER 23

Transplantation of Fascia in Humans

Articles describing the clinical use of free autogenous fascia grafts in humans are not found in the literature until the year 1905. Kirschner (1911) was probably the first to transplant fascia and *later expose the grafts and observe that grossly the transplants had not been absorbed and that they appeared exactly like fascia.* He believed that the transplants survived as such.

Denk in 1912 removed a free autogenous fascia transplant and, histologically noting broad bands of host fibrous tissue invading the graft, stated that fascia grafts were gradually replaced by host tissue.

Thus arose the two conflicting viewpoints which are found throughout the literature and still exist regarding the behavior of autogenous fascia grafts. All agree that fascia grafts retain their specific structure as fascia following transplantation. Some authorities, however, feel that the cells in the grafts survive, whereas others, subscribing to the host tissue replacement theory, believe that host fibroblasts gradually infiltrate the graft and replace the original cells.

It is intertesting to find that exactly the same controversy also exists regarding the behavior of free autogenous tendon grafts.

REVIEW OF LITERATURE ON FASCIA TRANSPLANTS

The first instance of fascia transplantation in the human is attributed to Bruns (1), who in 1905 applied a band of fascia lata for ligation in prolapse of the rectum.

Kirschner (1911) is said to have been the first to suggest that fascia might be used to repair defects in the tendons (2). On the basis of a large series of operations on humans, he was able to establish that transplanted autoplastic fascia, even in large transplants, remains as fascia and heals in organically without reaction (3).

At a German society meeting in 1908 Payr (4) presented a patient in whom he corrected congenital ptosis of the upper eyelid by anchoring a free autogenous transplanted band of fascia lata above to the muscular frontalis and below to the tarsus. After a half year the result was still satisfactory from the functional and cosmetic viewpoints; the patient could open and close the lids the same on both sides.

Koenig (1909) is said to have been the first to use fascia to bridge defects in the walls of hollow organs and to strengthen doubtful suture lines (5). He thought the procedure useful in abdominal operation but considered it not as a permanent cure but only for alleviation. In his opinion the factors in success were extensive mobilization, careful adaptation of the widest possible wound surfaces, and perfect asepsis. In one case of recurrence of abdominal hernia after closure four times, a flap taken from the anterior tibial surface, including periosteal

strips and a thin layer of corticulis, was laid over the suture line, with the bone inward and fixed with suture. The wound healed well, with firm apposition (6).

By inserting chromicized pig's bladder into ankylosed joints Baer (7) of Johns Hopkins University (1909) was able to obtain a definite amount of permanent mobility provided the membrane could be retained for a period of 30 to 40 days. In the reported cases, ankylosis developed from gonorrheal arthritis, septic arthritis of the knee, tuberculosis of the knee and tendon sheath of the wrist, tuberculous arthritis of both hips, synostosis of the elbow, and tuberculosis of the knee and of the hip. In each patient the permanent motion was of definite utility.

Rothchild (8) (1910) transplanted a piece of autogenous fascia lata to correct a functional disturbance due to paralysis of the trapezius muscle. The fascia lata was sutured to the vertebral border of the scapula and to the supraspinous ligament, correcting the dropped shoulder successfully.

In 1911 Wilms (9) performed an occlusion operation of the pylorus on a human, utilizing free autogenous fascial strips.

As reported by Denk (1912) a sheet of autoplastic fascia lata was used to cover a defect caused by removal of a tumor from the parietal bone, with dura. At a second operation in about a year the recurrent tumor was extirpated with the fascia covering, and a new fascial flap placed over the defect. Two months later the patient was well. At histologic examination the implanted fascia showed broad connective-tissue fibrils in parallel arrangement, with sparse, well-stained nuclei. Vessels and elastic fibers were present in a relatively large quantity (10).

In a second case Denk was able to demonstrate the penetration of granulation tissue between the fascia bundles after the fifth day, and he concluded from a study of the two cases of dural defects that fascia does not heal in as such but is replaced by scar connective tissue (11).

Denk commented: "The free fascia transplantation suggested by Kirschner has surmounted the experimental stage and has become the common property of operative surgery." He reported that such transplantations had been performed nineteen times at the University Clinic in Vienna to cover brain defects, for repair of joints, strengthening abdominal suture after ventral hernia, as intestinal suture after rectal resection, and for bridging urethral defects. In his opinion, fascia almost always heals in aseptic fields. Denk advised that care be exercised to have the fascia come in contact with the nourishing tissue parts in as large a surface as possible. The danger of muscle hernia at the site of fascia removal is relatively slight. If it does occur, it is to be regarded as harmless (12).

Von Tappeiner (13) (1912) employed autogenous facial band for occlusion of the pylorus in three patients, the longest period of observation being two months. He considered it to be the only satisfactory procedure.

Transplantation of autogenous fascial flaps from the fascia lata femoris was first carried out by Levit (14) (1912) to cover a tracheal defect in a patient. Eight months later the patient was completely well; the graft held perfectly. There was no pain in motion or in breathing.

Kornew (15) (1913) implanted an autograft of fascia into a pleural defect and examined a section at the end of a year. The physical structure of the transplant was retained in a general way although it had undergone some alteration. In his histological report it is stated that the transplanted tissue is intimately interwoven with the surrounding tissue, its transversely divided bundles being prominent, and the longitudinal ones poorly defined. Under higher magnification a dense layer of connective tissue is present on the superficial surface of the tissue adjacent to the fascia. Elastic fibers are present to the same extent as in the

neighboring well-defined fascial layer. The tissue within the fascia has a definite fibrous character, with well-defined development of blood vessels. The outline of the fascial layer transversely sectioned is clearly visible and in the central portion of the layer "the infiltration of younger elements" is hardly perceivable. A thin layer of undulating connective tissue containing many capillaries lies between the two layers of transversely divided bundles.

Toward the pleural surface there is present an inner longitudinal layer not as wide as the transversely divided bundle but having a similar tendinous structure with well-stained nuclei. In some areas the fibers are separated by younger connective tissue which fuses with scar tissue toward the pleural surface. Unlike the outer part, the fibers in the inner part of the transplant run in various directions and do not have well-defined limits. Numerous elongated and oval cells with large nuclei are present.

(Neuhof saw no conclusive evidence that the tissue described by Kornew as fascia "is not really a layer of connective tissue that has completely replaced the original transplant."[1])

Before a meeting of the Greifswald Medical Society, in 1913, Payr (16) presented a patient who had suffered a wound, with infection of the tendon sheath with loss of the flexor tendons, and subsequent contracture of the finger. After the flexor tendon stumps had been freed of surrounding scar tissue, the finger was extended. A piece of fascia from the thigh was folded cylindrically and established as a flexor tendon to the periosteum of the terminal phalanx and to the flexor tendon stumps. Active bending resulted in the hand, and was possible in the first interphalangeal joint to a somewhat less extent. Shortening of the constructed tendon was to be expected.

Chiari (17) (1913) reported a case in which a tumor was radically removed from the frontal bone and the dura was covered with an autograft of fascia from the thigh. The wound healed but in a second operation for recurrence some weeks later implanted fascial pieces were recovered and showed blood pigment residues, and swelling and liquefaction of individual fascia bundles. Contrariwise, large sections remained alive, as shown by good staining of the tissue and cell nuclei. The fascia appeared to form a foundation over which the advancing granulation tissue spread from the edge, providing nutrition. Additionally, the connective tissue soon supported the fascia in maintaining firm closure, thus forming a resistant wall. The disturbances and changes in the finer structure are explainable, on the one hand, by the unavoidable operative injury, and on the other hand, by the adaptation of the living fascia to the changed conditions of nutrition and other mechanical factors. Dead fascia naturally would not be capable of such adaptation.

In a case reported by Giertz (18) (1913) free autogenous aponeurosis from the thigh was transplanted between severed tendon stumps of the thumb. In another patient with contraction of the thumb from suppuration in childhood the scar was removed and autogenous fascia lata from the thigh sutured between the tendon stumps. Autogenous fascia lata was employed as a collateral ligament after suppuration of the knee joint in another patient. In all three patients the fascia healed in, and served as an excellent replacement material, even in very long pieces.

Neudörfer (19) (1913) obtained good results in spina bifida occulta and in meningocele occipitalis inferior in small children by the use of autogenous fascia grafts.

After this variety of restoration with au-

[1] The elastic fibers which were noted in the graft structure were undoubtedly the original ones present in the transplant because the production of new elastic fibers after they have once been formed has never been demonstrated in humans.

togenous fascia lata transplants it is not surprising to find McArthur (20) (1913) expressing the belief that tissue transplantation is a "live and growing" topic. *He pointed to the microscopic demonstration that "cells supplied with sufficient appropriate plasma, under conditions approximating those of life, will live and multiply, each retaining its own particular characteristics."* From the repetition of cases and testimony, so he remarked, "one finally realizes that under aseptic conditions almost any autogenous graft may remain viable in any other part of the donor's anatomy."[2] McArthur suggested two operative steps in the taking of the tendinous strip for transplantation: first, loosening and raising of the desired fragment from its normal position, then burying it in the neighboring subcutaneous fat for a period of two weeks or more, thus allowing it to be healed in. Secondly, after a time the strip should be dissected out with a covering of fatty tissue adhering to it.

A series of free autogenous fascia transplantations were carried out by Thäle (1913) to bridge defects in the abdominal wall, in joint ankylosis, and to replace tendon in injuries of the hand, all being attended with success (21).

Kleinschmidt (22) (1914) recognized the advantages of fascia in clinical application. One practical value was the availability of a living autoplastic aseptic material in an almost inexhaustible supply. Fascia has great firmness and yet its elasticity is such that it easily assumes all possible forms. Thus, there is possible adaptation of this living material to all forms of organs. Kleinschmidt held that since the discovery of its utility free fascia transplantation has gained a wide domain of application, as demonstrated in the large number of publications concerned with such autoplastic material. He foresaw that many more procedures concerned with fascia transplantation would be introduced in the course of time.

[2] McArthur in 1913 made a statement which, excepting for free muscle grafts and bone, is possibly more accurate than many later statements by others on the behavior of free autografts. His idea of first transplanting the tendon graft in fat is interesting.

In a review of the literature on free transplantations of aponeurosis, Greggio (23) (1914) found that aponeurotic fragments had been used in dural plasty, in closure of cerebral ventricle, in occipital meningocele, in tracheal wounds, in plasty of the thorax and in pulmonary hernia, in radical treatment of umbilical lateral postlaparotomic hernias, in inguinal and large crural hernias, in wounds with loss of substance of the diaphragm and in intestinal fistula, and additional conditions. *He felt, however, that the indications for the use of free aponeurotic grafts had been exaggerated, as often happens at the beginning of the employment of a new operative method.*

Greggio himself employed free transplantation of autogenous femoral aponeurosis in some patients with recurrence of inguinal hernia when the abdominal wall, at the level of the hernia, was insufficient for radical treatment because of the state of the muscles. He utilized large bands of free aponeurosis successfully in radical treatment of umbilical hernia, and in a patient with considerable loss of substance of the abdominal wall who had been operated on six times by other surgeons without success. In these latter cases the results were excellent in 12 to 15 months after operation.

In a case of large ventral hernia with numerous weakened areas in the lower half of the abdominal wall, Shaw (24) (1915) sutured an autogenous iliotibial band of fascia lata of immense size (23.5 by 13 cm.) under great tension to the oblique muscles externally, to the recti and oblique muscles above, and to the pubis and oblique muscles below. Perfect success was obtained in spite of infection with partial slough, and poor nourishment. Shaw held that for the first few

days a transplant must obtain its chief nourishment from lymph exudate supplied by the surrounding tissues, and eventually from vascular connections. Therefore, the less vascular the transplant is and the more vascular the soil, the greater is the assurance of success. Autoplastic fascia transplants undergo practically no histologic changes.

In a society report at the New York Academy of Medicine in 1915, Neuhof (25) presented a patient who had been under observation for 9 months after an autoplasty. A piece of autogenous fascia lata was sutured in a tracheal defect. Though there had been leakage and extensive emphysema, the results were good. In another patient autogenous fascia lata had been sutured over a defect covering more than half the perimeter of the urethra, with a perfect clinical result in a year after operation.

In a German publication in 1916, Stein (26) stated that for years he had carried out the closing of fascia lata in all fascial removals for autoplastic purposes, with excellent results. Primary healing of the wound took place in all patients, and no muscle hernia was ever observed. In other words, no ill-effects resulted in the donor area of the thigh.

In treating war wounds (in World War I) Burk (27) (1916) made transplantations of free fascia autografts with varying degrees of success. A fascial strip replaced a relaxed and torn deltoid ligament, and paralyzed flat foot was corrected by suturing the tendon of the paralyzed tibialis anticus through the canal to the tendon end. For the protection of the brain, fascia was transplanted to cover a skull defect. In total facial paralysis one end of fascia lata strips from the thigh was anchored to the angle of the mouth and the other end to the middle of the upper and lower lip and to the triangularis muscle. In absence of dorsal flexion of the hand joint, the muscle septa were replaced by free fascia, resulting in good movement of the hand joint. In stiffening of the hand joint an excellent result was obtained by an autograft of fascia.

Hoffmann (28) in 1916 made the statement that to his knowledge a number of surgeons do not close the defect caused by the removal of fascia from the thigh. He believed that even large defects, which apparently could not be closed, with good technique could be approximated.

Still another publication appeared in 1916, namely, Wierzcjewski's, in which he expressed the belief that without mechanical demand fascia maintains its structure but it shrinks about one-fifth to one-sixth in volume, as seen in transplantations. In one of his patients with injury of both hands, twelve pieces of fascia were removed from the thigh without functional disturbance occurring in the legs. Wierzejewski had an opportunity to establish *that defects in fascia in the thigh donor area were later compensated for through new fascia formation*, which was differentiated by reticulated fibrillation from the original tissue. He believed that fascia played a large role in the fixation of joints, especially in habitual luxations of the shoulder and knee. He used free autogenous fascia transplantation in the building of a stump in an amputation of the forearm, and also in the use of a prosthesis with good clinical results (29).

The wide application of fascia transplantation is evident in a report by Stewart (30) in 1917. He stated that he had transplanted completely detached pieces of autogenous fat and fascia about one hundred times. Emphasis is placed on the observation that in the operation for hernia, and in arthroplasty the danger of acute necrosis of a free transplant is little or no greater than the danger of suppuration in the same operation without free transplantation.

In a case reported by Neuhof (31) (1917) a tracheotomy had been performed ten years previously and the wound never closed. The

patient requested relief chiefly because of the cosmetic appearance and difficulty in phonation. The defect in the trachea was freshened and a slightly larger sheet of autogenous fascia lata of the same shape was removed from the lower thigh and transferred immediately to the defect. The muscle surface of the transplant faced the lumen of the trachea. The fascia was turned to the tracheal wall and sutured. Primary union took place; phonation was good. The cosmetic result was satisfactory. Neuhof considered that free fascia transplant in aseptic areas has a wide field of usefulness.

In a boy with a urinary leakage from the undersurface of the penis since birth, Neuhof placed a section of autogenous fascia lata, removed from the thigh, in the defect. The fascia was fixed in place with the muscle surface toward the lumen and the margins slightly overlapping the margins of the urethral defect. Urination was normal one month postoperatively. The wound was firmly healed. There was no evidence of stenosis; the results were good.

Dean Lewis (32) in 1917 expressed the opinion that in certain types of plastic surgery both fascia and fat have to be transplanted together or the fat must be transplanted first to prepare for the direct transplantation of fascia. He considered fascia an ideal material for transplantation, for being thin it is easily permeated by serum, and it undergoes little or no degenerative changes after direct transplantation.

In a case of neurolysis Lewis covered the line of suture fixing a resected musculospinal nerve with a fascia graft. The return of function was almost complete. Nerve regeneration after tubulization shows protoplasmic band formation preceding the downgrowth of axis cylinders from the central end and entering the bands, which act as conduits for the developing axis cylinders. The transplanted fascia in some instances has become almost undistinguishable from the perineurium.

Balleuil and Jack (33) (1918) used autogenous fascia lata successfully for painful adherent scars.

In 1921 Gallie and Le Mesurier (34), after a report on their animal experiments, pointed out the clinical value of the principle of the use of living sutures of tendon and fascial strips. The edges of the gap in the fascia hold together solidly after operation. They attributed to insecure union most of the failures that have attended so many of the operations in which transplanted fibrous tissues have been used, and *they emphasized the value of overlapping the fascia to provide a broad strong surface for attachment.* The various fields in which the living suture method has been or may be employed are indicated, and examples are given to demonstrate its clinical value.

To illustrate the applicability of fascia lata, Cuff (35) (1922) presented various cases of reconstruction, which "should be based on strictly anatomical lines wherever possible." Fascia lata from the thigh was transferred over a deficiency in the oblique muscles from the rectus sheath to the remains of the external oblique aponeurosis, to the extensor communis digitorum and the longus pollicis; similarly, to the periosteum in a wound of the left shin with a scar. The results were satisfactory also from transplanting a bone graft from the tibia in a gutter of the mandible and then wrapping fascia lata around the graft covering the periosteum of the transplant. A lipoma over the right deltoid was inserted into a defect from hernia of the right lung, and over this a fascia lata sheet was sutured to the periosteum of the ribs. In a patient with drop foot, fascia lata from the thigh was tunneled to the periosteum of the tibia and fibula and to the split aponeurosis down to the wound; the patient walked well after healing had occurred.

Neuhof (36) in 1923 maintained that in

not a single instance had transplanted fascia been extruded, not even in patients in whom infection had been present or had appeared postoperatively.

Koontz (37) (1926) suggested using alcohol-preserved strips of fascia lata from the ox in place of living human tissues. In experimental repair of hernia he found that the replacement process of living autogenous fascia took place in a manner analogous to that in dead ox fascia preserved in alcohol. *"The dead cells of the graft have been replaced by cells from the host . . ."*

Campbell (38) (1926) described a procedure in which a strip of fascia is passed around the exposed vessel in aneurysm and the margins approximated by suture at any required tension. The long end may be carried over the line of sutures and secured by lateral stitches, thus lessening the danger of hemorrhage. He operated by this procedure on the common carotid in three patients, and on the popliteal artery in a fourth patient, with perfect results.

Having demonstrated by animal experimentation successful transplantation of dead fascia grafts, Koontz (39) (1927) operated on 17 patients with hernias, using alcohol-preserved strips of fascia lata from the ox as suture material and employing the technique advocated by Gallie and Le Mesurier, with linen thread instead of catgut for transfixing the ox fascia. Healing occurred by primary intention in all the patients with the exception of two, in whom infection developed. Koontz suggested other uses for dead fascia grafts: for strengthening the capsule of relaxed joints, in bridging tendon defects, and in curing aneurysm.

Orrin (40) (1928) aptly described the transplantation and use of fascia lata from the thigh as a plastic method to correct deformities from extensive fibrous formation and scars and to prevent their occurrence "in imitation of Nature's own architecture." In his opinion, fascia transplantation has been used most successfully in diverse ways to reinforce or replace ligaments, tendons, and paralyzed muscles.

In discussing fascia graft for dislocation of joints, Sterling Bunnell (41) considered fascia transplants as the ideal method, for "it is a natural binding agent, it hypertrophies under conditions of normal tension, allows normal motion in the joint *and lives as long as the patient.*"

After a critical survey of the data on free fascia transplantation, Tschmarke (42) (1933) questioned whether free transplanted fascia has a general tendency to secondary shrinking. Based on his own investigations his decision was positive. The demonstrated shrinking tendency was traced back to the injury of the transplanted tissue caused by surgical manipulations.

Glasser (43) (1933) held that the great tensile strength and probable non-absorbability of ox fascia make its use valuable. Its advantage over live fascia is evidenced, since the results are equally good with both types, and ox fascia is more readily available. Failure to remove fat and areolar tissues from the ox fascia gives poor union. In 30 cases followed up from 6 months to 2 years, the clinical results were excellent.

Gilcrest (44) (1934, 1936) estimated that the tensile strength of the biceps is about 150 pounds. If rupture is at the upper or lower musculotendinous junction, fascia transplants and sutures should be employed. When the rupture is in muscle, the defect should probably be overlapped with a fascia transplant in addition to suture for purposes of reinforcement.

Lowman (45) (1936) considered autogenous fascial bands serviceable in bridging over weakened areas in the abdominal wall. All patients who had abdominal wall transplants showed some improvement in physiological function. In the 35 patients operated on, the transplants were usually done as

tendinous extensions from muscle to bone, but sometimes from bone to bone.

In operating for hernia Gallie (46) (1936) weaved fascial suture back and forth. When exposed in recurrence the suture was found to be glistening and white, rounded in the form of thin tendon, and adherent to the structures through which the living suture passed; it was evidently composed of normal connective tissue and fascia.

Carrell (47) (1937) described his experience in securing stability in repair of the anterior crucial ligament and also support for hypertension weakness by the use of fascia lata strips from the thigh. The same type of plastic material was also employed in satisfactory repair of the medial ligament, with good fixation.

The greatest difficulty in using fascia transplants to repair weak or paralyzed muscles was found by Dickson (48) (1937) to be the accurate evaluation of what muscles or groups of muscles are chiefly at fault. He tried to replace lost action or recapture stabilizing influence by attaching fascia transplants from unparalyzed muscles to a fixed bony point. Benefits were derived from fascia transplants in plastic repair of impaired abdominal, superior rectus, inferior rectus, upper oblique, lower oblique, quadratus lumborum muscles and various combinations, as well as muscles concerned with shoulder action.

Brinizer (49) (1940) reported on the use of fascia grafts under bone grafts in skull defects, to pad cheeks, as bands to support the cheek and ectropion of the lower eyelid, between the mucous membrane of the face and cheeks for support, in bilateral and single harelips and in cleft palate to relieve tension, and to cover resected joint ends. Additionally, fascial flaps and sutures were employed in bone grafts and in repairing the abdomen, especially in hernias. In the maxilla fascial bands were placed between the mucosa and the muscularis to relieve tension and for reinforcement. The patient himself, Brinizer pointed out, offers "a handy and effective material in his own skin and fascia" to relieve these various deformities.

In experience with the removal of fascia lata in about 300 individuals Foshee (50) (1943) observed a bulge of thigh muscle for a few months, *which disappeared after six months in every patient who had been followed up*. He made a number of observations on the reconstructive processes in fascia. *Fascia lata regenerates to fill a defect* in a donor area where fascia has been removed. When every bit of fascia has been removed, new fascia lata of one-half to two-thirds of the thickness and tensile strength of the previous fascia lata regenerates to cover the whole area normally covered by this tissue. Moreover, this may well be used again for fascia sutures and transplants. This regenerated tissue "serves all the purposes of true fascia lata" in making a sleeve for muscles and in preventing herniation.

A fascial patch transplantation was used by Singleton and Stehouwer (51) (1945) for hernia in 175 patients during the years 1916 to 1944, with reliable periodic examinations postoperatively in 129. An autogenous fascia graft, from which the loose areolar and fatty tissues had been removed, was transferred to the inguinal wound with its upper edge placed beneath the muscle and sutured to the undersurface of the internal abdominal oblique and transversus muscles. In six patients who were reoperated on, the grafts showed no absorption. Clinical failure was due to inclusion of the areolar tissue in the plane of union between the graft and tendon, with excessive stretching or insufficient area of contact between the graft and its bed.

Pieces of ox fascia preserved in 70 per cent alcohol were implanted into human tissues and studied by Chandy (52) (1946) over a period of four years. The preserved fascia rapidly became adherent to the human tis-

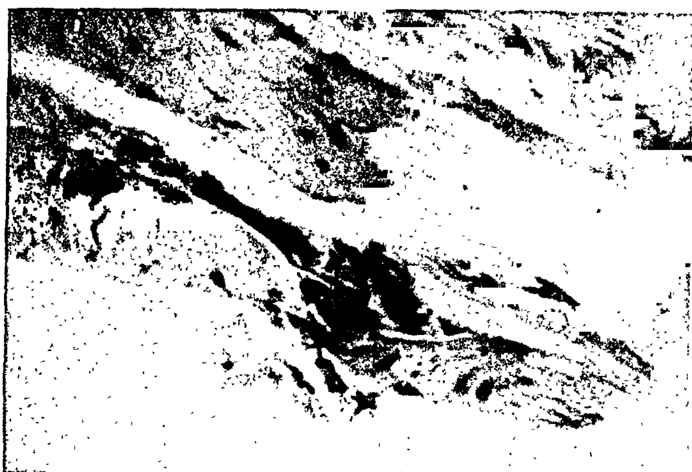


Fig. 93. Autogenous human fascia graft buried for 24 hours in abdominal fat. Note normal appearance of fascia cells and matrix. A small empty blood vessel is seen. Anastomosis between host and surviving graft blood vessel has not as yet occurred and the cells are kept alive by diffusion of host tissue fluid, or the centrally located cells merely maintain their life until the circulation arrives. X340.

sues and remained an integral part for a long period of time. It was gradually replaced by the ingrowth of fibroblasts and capillaries and the formation of new human connective tissue. After four years the ox fascia could still be identified. There was complete absorption of preserved fascia cells at the periphery of the graft. Fibers of the fascia became rearranged in bundles as more fibroblastic and capillary ingrowth took place. Chandy held that the preserved tissue does not in itself become a living tissue at any time. When tissue is dead, its intracellular chemistry undergoes changes and the devitalized material is absorbed and replaced by other tissue cells. The absorption may be slow if the material has been fixed in alcohol. The amount of foreign-body reaction depends on the clinical and physical nature of the foreign body. The fixed connective-tissue cells, not being irritating, do not necessarily have to produce such a reaction. There is, however, sufficient phagocytosis for the absorption of the foreign cellular detritus. He advises the application of ox fascia as a framework in plastic repairs.

In 1947 Foshee (53) reported on a study of regenerated fascia lata removed from five patients operated on for repair of hernia with free fascia grafts. Specimens of regenerated autogenous fascia lata that had been used in hernial repair were removed from transplantation sites at periods of 8 months, a year, 2 and 15 years, by permission of the patients or during a subsequent surgical intervention for recurrence. Foshee found that regenerated fascia lata contains two layers—an inner and an outer transverse layer. The prominence of the thickness and density of the inner transverse layer over the outer layer strongly suggested the importance of the proximity of muscle for the success of fascia regeneration. The activity of the individual seems to be a factor in the density and development of the regenerated fascia lata.

As expressed by Barrett Brown, McDowell and Fryer (54) (1948), the use of fresh autogenous strips of fascia lata continues to be one of the best methods of support for facial paralysis. Fascia is looped to the upper lip, the angle of the mouth, and the lower lip, and is anchored in the temporal muscle and fascia. The loop through the lower lid is anchored in the opposite frontalis region and in the temporal fascia.

Gallie and Le Mesurier (55) (1948) *stated that experimental studies on animals and clinical experience with humans demonstrate that fascia lata, as an autogenous graft, will continue to live unchanged.* They noted that the grafts always appeared grossly like fascia in those patients on whom they were obliged to operate a second time. Microscopic examination of a fascia lata graft which had been inserted six years before showed normal ar-



FIG. 94. Autogenous human fascia graft buried for 28 hours in abdominal fat. Note collapsed blood vessel with surviving endothelial cell lining. The fascia cells are normal in appearance on the basis of other sections, and there is no invasion by host tissue fibroblasts. There has been some diapedesis by graft white blood cells into the interstitial spaces of the graft substance. These white blood cells identified as polymorphonuclears and lymphocytes were "trapped" in the transplant when it was severed from the donor site. ×340.

rangement of the collagenous fibers and viable fibroblast cells. There was an absence of any cellular reaction in the graft.

Gallie (56) (1948) referred to an interesting case of a patient who had had a new inferior glenohumeral ligament made of fascia lata to prevent recurrence of dislocation of the shoulder. After ten years this new ligament was exposed and was found to have all the characteristics of a roll of fascia lata. He believes that if sheets of fibrous tissue such as fascia lata are transplanted to a place in the same individual where the blood supply is good, they will survive unchanged. As viewed by Gallie, it is necessary to overlap the edges for healing between the transplant and the host tissue, providing direct contact of scarified tissue.

Thatcher (57) (1949) considered fascia lata strips of particular value for replacement of injured flexor tendons in the hand when more than one finger is to be reconstructed or when the palmaris longus tendon is congenitally absent or inadequate. However, fascia lata strips should be used only in newly-formed tendon sheaths. The profundus tendon is dissected and the two insertions of the sublimis tendon are severed. The freed portions of the profundus and sublimis tendons are pulled out of the flexor tendon canal and discarded. A stainless steel rod bent to conform to slight finger flexion is introduced into the flexor tendon canal through the finger wound. As the rod is withdrawn, the fascia lata strip is pulled into the flexor tendon canal, the fat covered side of the fascial strip being turned outward.

In suspending a prolapsed kidney Strode (58) of Honolulu (1949) used fascia lata by weaving it in and out of the capsule of the lower pole. Satisfactory results were obtained in carefully selected patients with nephroptosis. The transplantation of autogenous fascia obtained from the leg or occasionally from the kidney incision itself gave gratifying results in his hands.

The author (59) buried autogenous fascia lata grafts in human abdominal fat and removed the grafts at 24-hour intervals over a period of 1 to 6 days inclusive. Grafts were also removed at 15 and 18 days, and at 4 and 18 months. The fascia grafts were all in separate transplantation sites and were buried in patients who volunteered as experimental subjects.

The grafts buried for 1 and 2 days showed extravasation of red blood cells about the graft and a beginning collection of cellular exudate outside the graft, associated with dilated host blood vessels and proliferation of host fibroblasts. The sparse blood vessels in the fascia grafts were collapsed and empty, or partly filled with cellular debris. Red blood cells were present in some of the small blood vessels and these appeared well preserved in the 1-day sections but were often

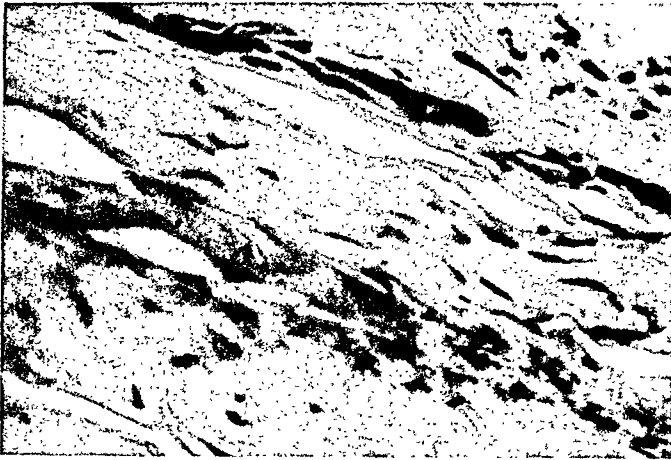


FIG. 95. Autogenous human fascia graft buried in abdominal fat for 3 days. In this graft the blood vessels are still collapsed and empty, indicating that anastomosis between host and graft blood vessel has not as yet occurred. The graft fibroblasts are more numerous than is usually seen in normal control sections of fascia. The collagenous matrix is edematous but otherwise normal. The endothelial cells lining blood vessels and the structure of the vessels appeared enitrely normal. ×340.

clumped and formless in the sections buried for 2 days. These red blood cells had apparently been trapped in the blood vessels at the time of transfer. A few polymorphonuclears and other white blood cells, which had been trapped with the red blood cells, had migrated through the vessel walls into the matrix of the graft. The endothelial cells lining the graft blood vessels appeared entirely normal. The grafts were edematous but the fascial cells showed no evidence of degeneration. The exudate was more marked in the host tissue around the graft buried for 2 days, and there were very many polymorphonuclears and lymphocytes present. *Mass infiltration by host fibroblasts into the structure of the grafts was not evident.*

One of the grafts buried for 3 days showed an intense cellular reaction both outside and inside the graft. Blood vessels within the graft were distended and filled with red blood cells and exudate cells, which in some locations could be seen in the process of diapedesis through the blood vessel walls into the interstitial substance of the graft. Endothelial cells lining blood vessels appeared normal, and apparently circulation had been established by end-to-end anastomosis between graft and host blood vessels. The fibroblast cells in the graft appeared normal but were quite numerous. Considerable edema was present but there was no mass invasion of the graft structure by host fibroblasts. *Very definitely circulation had been established some time between the second and third day after transfer.* In a second fascia graft removed after 3 days there was very little reaction within the substance of the graft. *In this series of grafts circulation was not established until the end of the fourth or fifth day,* and cellular infiltration of the graft structure through blood vessels was quite minimal. The extent of this cellular infiltration seems to vary in different transplants. It is not extensive, however, until blood circulation is established.

Other grafts buried for 4 and 5 days were quite edematous, and dilated blood vessels in the fascia transplants were engorged with red blood cells and many leukocytes. There were dense collections of cellular elements in some areas of the fascia; here polymorphonuclears and lymphocytes could be seen in process of passing through the blood vessel walls to join others already in the interstitial tissue spaces. Plasma cells and eosinophiles were also present in the exudate. The fibroblasts appeared normal but had dark-stained nuclei and seemed about twice as numerous as the fibroblasts noted in control fascia. It seemed that the fibroblast cells had proliferated because of their increased number and active appearance, *but mitosis was not actually observed.*

In the fascia graft buried for 6 days the edema had largely subsided, the fascial cells

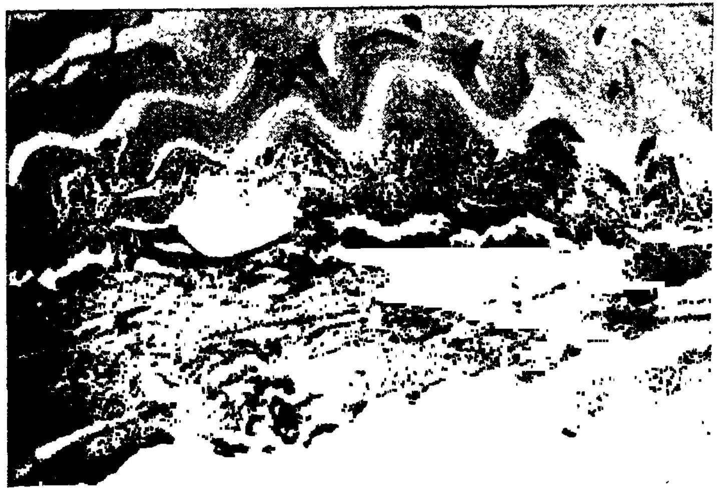


FIG. 96. Autogenous human fascia graft buried for 4 days in abdominal fat. Note blood vessel containing well-formed and normal-appearing red blood cells. Anastomosis between host and graft blood vessel has now occurred, and the critical period for survival or death of the cells in the graft has passed. Fascia cells and collagenous matrix are normal. ×340.

appeared to be viable, and there was no evidence of invasion of the graft structure by host fibroblasts. The endothelial cells lining blood vessels in the graft had a normal appearance and the vessels were dilated and contained numerous red blood cells and white blood cells. Cellular exudate within the graft structure was less evident compared with earlier transplants.

Fascia grafts buried for 15 and 18 days showed an absence of edema, only moderate cellular exudate within and around the graft, numerous normal fibroblast cells, and no evidence of invasion of the graft by host fibroblasts. The collagenous fibers appeared normal, and a fibrous capsule had formed about the fascia transplant. The blood vessels in the graft were still enlarged and distended by red blood cells, and the endothelial cells appeared entirely normal.

At 4 months the fascia graft had an entirely normal appearance except that there were about twice as many fibroblasts present as are seen in normal control fascia. The graft buried for 1½ years appeared exactly like the graft buried for 4 months. This graft also contained a large number of fibroblast cells. The appearance and arrangement of the collagenous fibers were identical to those of normal fascia.

The author's experimental work with preserved homogenous fascia grafts in humans indicates considerable reaction to the graft possibly on an antigen-antibody basis. Host blood vessels and fibroblasts *grew into the graft structure, and the fibroblasts and blood vessels of the graft disappeared.* Large cellular infiltrations of the inflammatory type were present within and outside the graft up to 8 months after transplantation. Possibly the grafts were in the process of replacement, but further experimental work is required to accurately determine the ultimate behavior or fate of preserved homogenous fascia grafts.

SUMMARY COMMENT ON FASCIA GRAFTS

It is interesting to note that the literature contains many references to autogenous fascia grafts and a fair number to preserved heterogenous ox fascia grafts, but there is little comment regarding the behavior of homogenous fascia grafts, either fresh or preserved.

Kirschner and later workers used autogenous fascia grafts and believed that they survived transplantation as living tissues. Denk (1912) and Kornew (1913) were the first recorded experimenters actually to remove an autogenous fascia graft and examine it histologically. On the basis of a single examination Kornew stated that in general the transplant retained its normal structure but still did not appear entirely like normal fascia. Denk believed that his

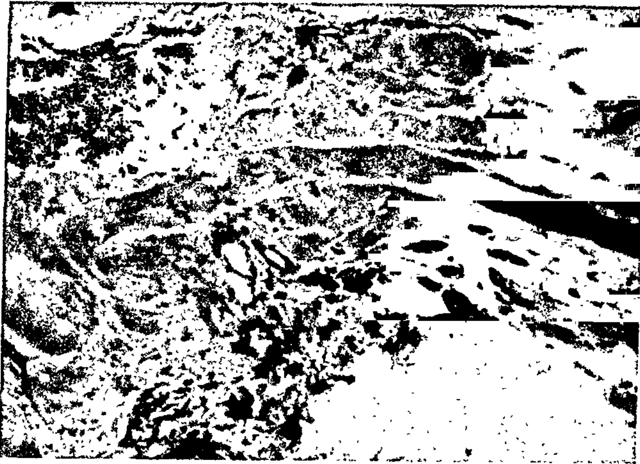


FIG. 97. Autogenous human fascia graft buried for 5 days in abdominal fat. Note normal appearance of fascia cells, which are quite numerous. Collagenous fibers are normal and other areas of the graft show normal blood vessels filled with blood cells. There is no evidence of mass invasion of host fibroblasts to replace fibroblasts in the graft. The fibroblasts in the graft appear quite healthy and require no such replacement. ×80.

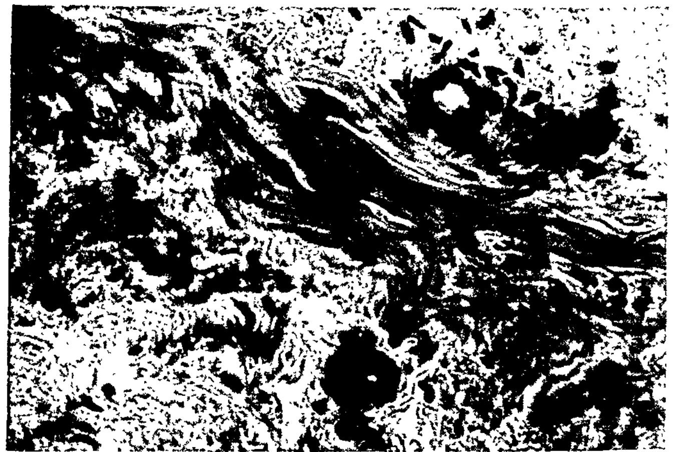


FIG. 98. Autogenous human fascia graft buried 18 months in abdominal fat. Note normal blood vessels, normal fibroblast fascia cells, which appear rather numerous, and normal appearance and arrangement of collagenous matrix. ×340.

fascia grafts had been replaced by host fibrous tissue.

Much of the later work was concerned with clinical results in humans and experimental work in animals. Many of these investigators believed that autogenous fascia grafts remained as such in humans, and they supported their statements by animal experiments in which autogenous fascia grafts were removed and examined histologically.

In careful experimnetal work with rabbits Gallie and Le Mesurier demonstrated that the fibroblast cells in autogenous fascia grafts remained viable after transplantation and that the graft retained its normal structure. In later work Gallie and Le Mesurier, and Gallie alone, made microscopic examinations of autogenous fascia grafts buried in humans for 6 and 10 years respectively, and noted that grossly and histologically the graft structure resembled that of normal fascia. This work is impressive not only because of the careful scientific methods used and described but also owing to the character of the men doing the experimental work. Le Mesurier is well known to plastic surgeons for his modification of the old Hagedorn operation for cleft lip repair, which is widely used both in this country and the world over. It is noteworthy that Sterling Bunnell in 1928 agreed with Gallie and Le Mesurier in the belief that autogenous fascia grafts remained viable following successful transplantation.

Neuhof, in numerous publications and in his fine book *The Transplantation of Tissues*, expressed the opinion that autogenous fascia grafts were extremely useful for clinical purposes. He believed, however, that host fibroblasts gradually infiltrated the struc-

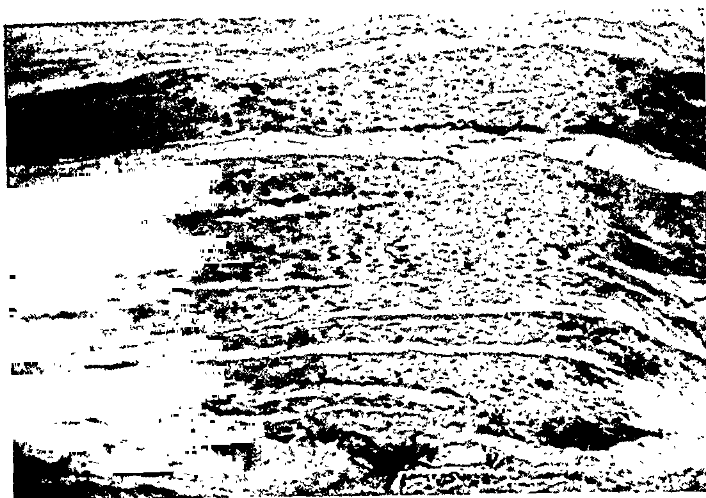


FIG. 99. Autogenous human fascia graft buried for 20 months in abdominal fat. See numerous fibroblasts and normal matrix. Grossly all fascia grafts appeared like normal fascia. ×80.



FIG. 100. Autogenous human fascia graft buried for 23 months in abdominal fat. This graft appears entirely like normal fascia excepting that it contains a large number of fibroblast cells. ×80.

ture of autogenous fascia grafts and replaced the original cells, which presumably degenerated and died.

Koontz, in 1926, and in later articles, also expressed the belief that the cells in autogenous fascia grafts were replaced by the host tissue cells and possibly the intercellular substance of the graft was similarly replaced although this is not specifically mentioned. This opinion represents the "high tide" of expression regarding host tissue replacement of autogenous fascia grafts, but it is important to note that *the theory is based on belief or opinion and not on actual facts.* On the basis of the meager or, more accurately, complete absence of factual evidence to support the theory of host tissue replacement, one is inclined to reject this hypothesis or accept it as a theoretical but unproved possibility.

Koontz in 1926 was one of the first to advocate the clinical use of preserved heterogenous ox fascia. He believed that ox fascia was just as good a grafting material as autogenous tissue, *since both were replaced ultimately by the host tissue.* Chandy in 1946 buried heterogenous ox fascia grafts, preserved in 70 per cent alcohol, in human tissues and removed the grafts for microscopic examination up to 4 years following transplantation. He found that after 4 years the ox fascia could be identified grossly as fascia. Examination of earlier sections demonstrated an ingrowth of host blood vessels and the presence of exudate host cells both within and outside the graft. He believed that the foreign graft was gradually replaced by the host tissue.

I have buried homogenous fascia grafts, preserved in 70 per cent alcohol, in humans

Fig. 101. A. Fascia graft (human autogenous) buried in fat for 8 days. Cells appear normal under high power magnification and there is no evidence of replacement from fibroblasts in the host tissue.

B. Fascia graft buried in fat for 1½ years. Structure is entirely normal.

C. High power magnification of fascia graft buried in fat for 1½ years. Note living fascia cells with their collagenous bundles.



Fig. 102. Boiled homogenous human fascia graft in contact with fascia buried for 14 days. There are numerous areas of liquifaction and general host cell infiltration including giant cells. ×80.

and observed about the same findings as found in the heterogenous grafts reported by Chandy. The oldest graft removed and examined microscopically in this series was buried for 8 months. In all of these sections of preserved homogenous fascia grafts the structure grossly resembled that of normal fascia. Microscopically, however, there was

a large amount of cellular exudate both in the graft structure and in the host tissues outside the graft. New host blood vessels and fibroblasts had grown into the graft, and there were occasional foreign-body giant cells within and outside the graft. In some areas it appeared that the collagenous fibers of the graft were in process of absorption.

My own experience with autogenous fascia grafts buried in fat indicates that the fascia cells in the graft not only survive but divide at least once, so that the total cellular population of autogenous fascia grafts is about doubled. This increased number of cells seems to remain up to 1½ years following transplantation, which was the period of burial for the oldest section examined.

Blood circulation in grafts is established in about three days after transfer, through direct anastomoses between host and graft

Drawings Indicating Usual Behavior of Fascia Grafts in Man

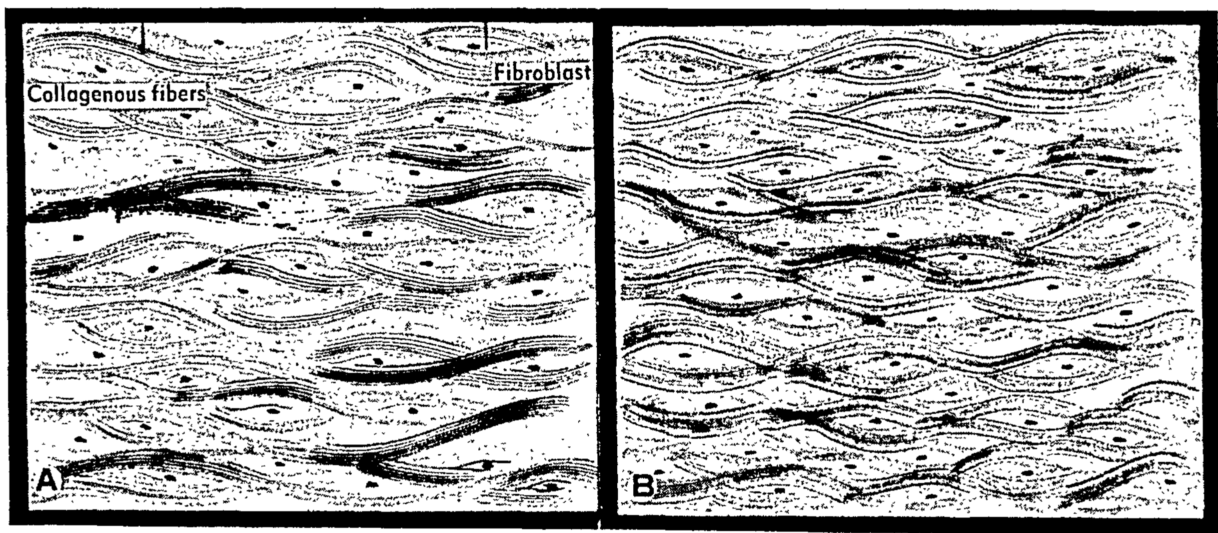


FIG. 103. A. Fresh autogenous human fascia grafts in contact with fascia. B. Graft remains as fascia. The fibroblast cells in the fascia graft remain viable associated with their collagenous fiber matrix.

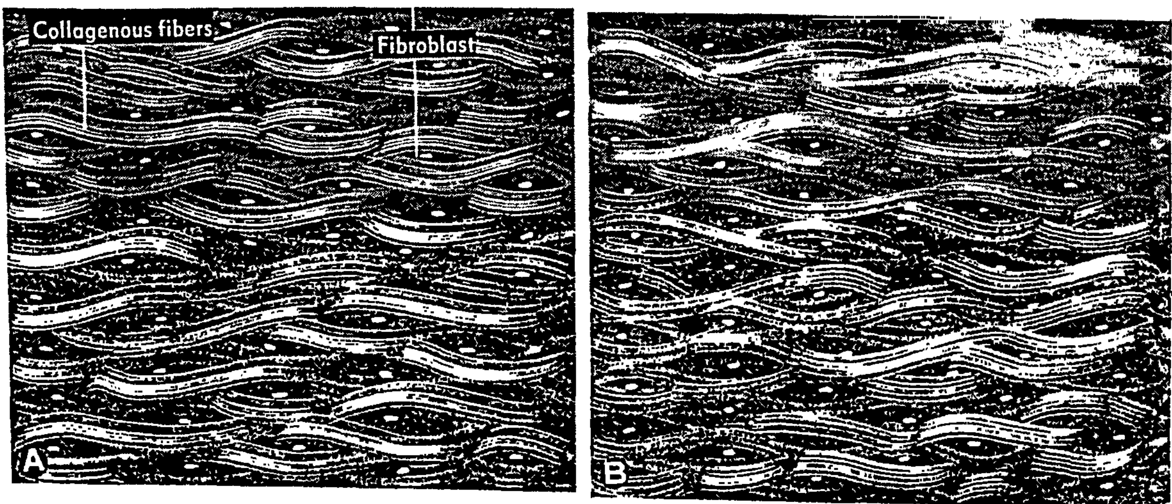


FIG. 104. A. Fresh autogenous human fascia grafts in contact with fat. B. Graft remains as fascia. The fibroblast cells in the fascia graft remain viable associated with their collagenous fiber matrix.

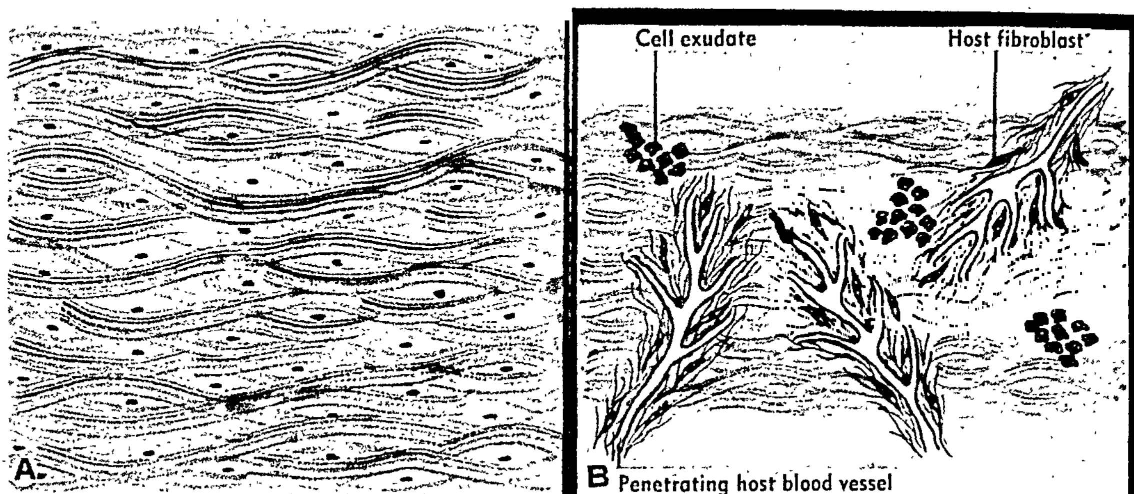


Fig. 105. A. Preserved or fresh homogenous fascia grafts in contact with fat or fascia. B. All living cells in fresh grafts fail to survive. The graft structure is invaded by host blood vessels accompanied by host fibroblasts. Collections of host cell exudate are scattered throughout the graft. Host fibroblasts, histocytes and occasional giant cells are also seen in the graft. The ultimate fate of the collagenous fiber matrix is not known.

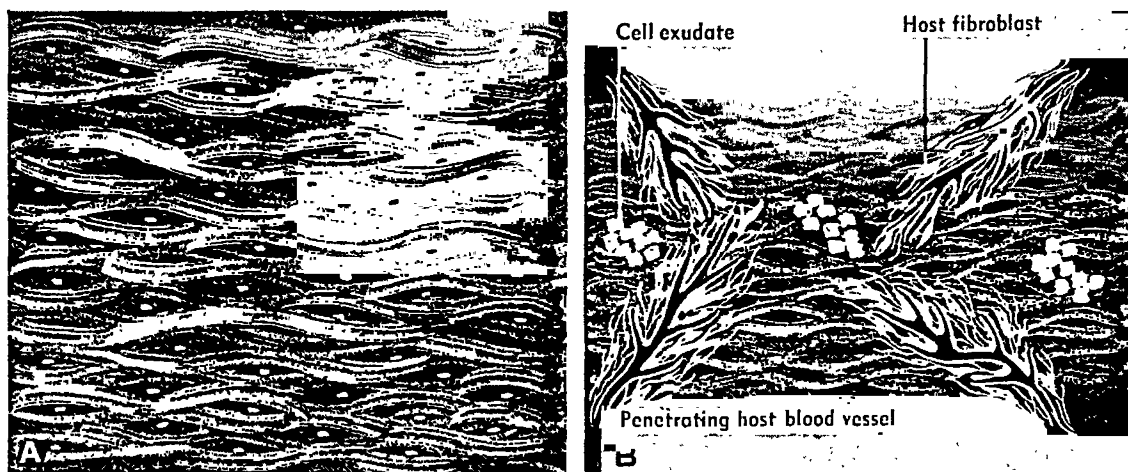


Fig. 106. A. Preserved heterogenous fascia grafts in contact with fat or fascia. B. The behavior of these foreign grafts in human tissues is similar to that of homogenous grafts excepting that the host reaction is more pronounced.

blood vessels. The vascular system with its endothelial cell lining survives in autogenous fascia grafts, and an excess blood supply may remain up to 4 months or longer after transfer as in surface skin grafts.

The initial edema and cellular exudate gradually disappeared between 6 and 18 days after transplantation, and the appearance and arrangement of the cells and collagenous fibers in the graft resembled those seen in normal fascia.

Mass infiltration by host fibroblasts into the substance of the graft was not observed at any time, and the positive evidence favors the interpretation that autogenous fascia remains as such after successful transplantation. The fibroblast cells in the graft remain viable and are not replaced by host cells. The behavior of autogenous fascia grafts closely resembles that of autogenous tendon grafts.

There is much to learn about preserved homogenous fascia grafts and heterogenous

fascia grafts from the ox implanted in human tissues. It is possible that these foreign grafts in contact with like tissue (fibrous tissue is present almost everywhere in the body) may be replaced by the host cells like homogenous and heterogenous bone grafts in contact with living host bone. Considering the availability of the patient's own fascia, however, it seems expedient to use only autogenous grafts.

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CHAPTER 24

Transplantation of Tendon in Animals

Although Galen differentiated tendon, nerves and ligaments anatomically, he considered tendon as consisting of nerves and ligaments. He advised against tendon suture because he believed that suture within nerve substance tended to be followed by severe pain, twitching, and convulsions, a dictum accepted for many centuries. However, the Arabian physician Avicenna advocated tendon suture, which gained acceptance by some Italian surgeons. Early in the fourteenth century the French surgeon Guy de Chauliac attempted the closure of tendon wounds with some success. About two hundred years later successful instances of tendon suture appeared in the works of Ambroise Paré (1).

AUTOGENOUS AND HOMOGENOUS TENDON GRAFTS

It was during the seventeenth century that animal experimentation with suturing of tendon was undertaken. Lanzweerde (2) (1655) related how he divided the tendo Achillis in a dog and sutured it. Healing occurred shortly, and the dog's movements were perfect. Nuck (3) divided the external flexor of the carpus of a dog and sutured the tendon, resulting in complete recovery in a few days. Meekren (4) in 1682 reported on the division of several tendons incompletely. Following the demonstration by Haller in the middle of the eighteenth century that tendons were insensible, operative procedures on them were generally accepted. From his experimental studies on dogs, Hunter (1767) concluded that tendon heals by the formation of callus in much the same manner as bone callus forms.

V. Ammon (5) in 1837, experimenting with horses and rabbits, held that the defect left by tendon injuries was first filled with a formless exudate (blood from the cut tendon ends and plastic lymph from the tendon wound), which formed a string-like white lymphatic structure. This united the tendon ends and consolidated into a tissue very much like tendon.

Both Pirogoff and Paget showed that the quick regeneration of a piece of tendon occurs without intermediate healing of the transplant (6). Investigations of the regeneration of the Achilles tendon after tendotomy in dogs, calves, and horses were carried out by Pirogoff[1] in 1840. He believed

[1] A year and a half before the Crimean War (in 1853) Nicolaus Pirogoff (1810–1881) began the treatment of fractures by using a plaster cast. He was the first surgeon in the world who used this method in military surgery and on a very large scale during the siege of Sevastopol.

His book entitled *The Principles of General War Surgery*, which was published in Russian and German in 1865, is a classical work on field surgery "without an equal in any country in the nineteenth century." (Surg., Gynec. & Obst., **78:** 7, 1944.)

that the extravasated blood in the tendon *sheath is indispensable for the formation of* intermediate substance. The reason is that if one prevents bleeding between the tendon ends, or presses out the blood from the puncture of the tenotome, then new formation of the intermediate piece does not take place or is incomplete (7). The importance of the lymph transudate in regeneration of tendon was also stressed by Duparc (8) in 1847. He divided the Achilles tendon in rabbits at different times and examined specimens microscopically. A specimen removed 3 hours after division showed interspaces between the tendon ends and extravasated blood filling the tendon sheath and cellular tissue, in which the cellular filaments were detached from the tendinous sheath. Following capillary and lymph plastic transudation, an amorphous substance, called "blastema," was present in the interspaces and at the ends of the tendon. After development of nuclei there was the formation of cells (cytoblastema) which divided into thin plaques. The fibers reunited in bundles which established the continuity of the cut tendon.

Paget (9) in 1853 also reported his observations on regeneration after tenotomy of the Achilles tendon in rabbits. The intermediate space was filled with fluid or simifluid substance which quickly became organized into forms of lymph or exudation cells. The extravasated lymph was converted first into a cartilaginous germ tissue and later into fibrous tissue.

The tendon in rabbits was divided by Adams (10) (1860), leaving a gap. Microscopic examination was made at intervals up to 62 days. Adams showed that divided tendons in the rabbit became reunited by a newly-formed connective tissue, which gradually assumed the structural character of the old tendon so perfectly that microscopically no difference could be perceived. A perfect reproduction of tendon took place in form, definition, and size, corresponding to *the tendon which it united. Externally* continuity became perfect after a few months, so that the line of junction could not be recognized. When longitudinal section was made, the line of junction of the old with the new tendon could be recognized at the latest period to which the experiments extended, i.e., on the 62nd day.

Dembowski (11) (1869) carried out 50 tenotomies on rabbits and followed the local histologic changes from some hours after operation to the formation of a complete scar. He believed that cell proliferation from the tendon sheath and from intertendinous tissue of the tendon stump took part in the formation of the scar. The presence of blood extravasation in the tendon sheath is important since it incites inflammation; the extravasation being gradually resorbed after the blood clot is organized.

Dembowski, Volkman (12) (1873), Billroth (13) (1882) and Güterbock (14) agreed in their belief that the character of the tendon sheath has a decisive influence on the reunion of resected tendons. Tendon tissue is capable of only a slight reaction.

Güterbock (15) in 1871 passed threads through the tendons of adult rats, rabbits, and guinea pigs and examined them from 2 to 24 hours after operation. In the treated sections the cells appeared severely swollen and assumed the form of a blackberry. He concluded that in inflammation the tendon cells participate actively in the proliferating process to a considerable degree.

Gluck (16) (1881) is said to have been the first to demonstrate that tendon could be transplanted directly. In experimental myo- and tenoplasty he transplanted the gastrocnemius tendon from one fowl to another and demonstrated that the function of the transplanted segment could be maintained. The tendo Achillis transplanted with the muscle had a perfectly normal gross appearance; examination of one transplanted tendon

being made after 40 days. No mention was made of histologic examination of the transplanted segments.

In experiments on frogs reported by Beltzow (17) of St. Petersburg in 1883 the Achilles tendon was incised lengthwise or transversely in one extremity, and completely resected in the other extremity, covered with skin and closed with silk sutures. Observations were made daily for 5 months after operation, the involved tendon being examined macroscopically and microscopically. Furthermore, normal tendon tissue was examined in the earliest embryonic stages to its complete development in an adult animal, in microscopic sections as in teased preparations.

At the end of the third week the tendon wound was filled with cells in layers between blood corpuscles—spindle-shaped cells, with granular protoplasm, arranged in parallel rows in the tendon tissue and projecting into the lumen of the wound. These newly-formed cells changed into cell elements of the thickened tendon sheath, which projected into the wound region. At the end of three months microscopically a white fibrous interstitial substance between the cells assumed a distinct fibrous character. Beltzow was convinced that the increase in tendon cells advanced toward karyokinesis (mitosis or indirect cell division).

Working with rabbits and guinea pigs he made an incision in one extremity and a complete section of the tendo Achillis in the other extremity. He found that the tendon tissue reacts energetically to an applied stimulus, and this reaction is more intense in warm-blooded than in cold-blooded animals. The reaction is entirely independent of inflammatory granulation formation under certain conditions. (The karyokinesis occurs in fixed corneal cells under stimulating conditions and in the tendons of the embryos of mammals under physiological conditions.) In Beltzow's opinion the cells with karyokinetic figures are true tendon cells, not migrating cells. Old tendon fibers take no part in the union. Although the substitute tissue is histologically almost identical with normal tendon tissue, it possesses the physiological properties of scar tissue. Regeneration of tendon tissue does not occur in the strict sense of the word.

In a series of experiments Fargin and Assaky (18) in 1885 transplanted pieces of homogenous tendon within the same kind of animal. In rabbits and guinea pigs a defect in the Achilles tendon was produced, and replaced by the tendon from the same species. Healing was reported to have been equally favorable in the different species. Wölfler (19) in 1888 claimed he was the first to carry out tendon plasty by implanting fresh animal tendon in animals, though he appears not to have publicized his experience.

Viering (20) in 1891 split the Achilles tendon of the rear extremity of rabbits and made observations in 2 to 50 days after operation. He believed that the connective tissue surrounding the tendon plays an essential role. The tendon cells were much enlarged, and the chromatin in the enlarged nuclei was modified. The presence of tendon cell mitoses was noted on the fourth day. Under functional stimulus the nuclei and the fibers of tendon arrange themselves in rows parallel to the line of tension. Viering's observations of the healing process confirmed Pirogoff's view that the scar forms very differently according to whether a large or small blood extravasation occurs in the defect between the stump ends at operation.

In Enderlen's (1893) study of the reparative process in the tendo Achillis of the guinea pig, the importance of the tendon cells in the regenerative process was demonstrated. First blood exudate forms in the gap, and there occur proliferative changes of the internal and external peritenoneum and of the surrounding connective tissue to some extent. Regenerative changes and mitoses

take place in tendon and in peritenoneum. These tendon mitoses do not depend on the proximity of blood vessels, as they are often seen at some distance from the vessels. Proliferating tendon cells migrate into the defect, and then tendon fibrils appear among the nuclei and form rapidly. By the ninth day there is union of the stumps. After the fifteenth day proliferation slows up; the fibrils form in a parallel arrangement and finally new tissue assumes the appearance of normal tendon (21).

In a series of experiments, Hoffa (22) (1901) studied the healing processes in dogs and cats in which the Achilles tendon and tendons of the paws were lengthened, reefed, or the tendons were laid on one another and sutured. Observations were made at varying intervals from 14 to 42 days. Hoffa concluded that the tendon tissue, peritenoneum and peritendinous connective tissue participated in the formation of scar. In the new formation of tendon tissue numerous bundles of young tendon arise that penetrate the scar and enter the inner retes with homogeneous fascicles.

Marchand (23) (1901) examined the lengthwise section of the Achilles tendon of the rabbit microscopically 15 days after sectioning. The fibers in an undulating course are enclosed in a thick fibrillar mass rich in cells. These cells, without a distinct outline, change into the thickened tendon sheath and connect with the widened bands of the intertendinous connective tissue. In the thickened sheath there are interlaid isolated residues of fibrin clumps. With stronger magnification the sectioned tendons show a much greater abundance of cells than the unsectioned. The bundles of fibers are loosened and especially separated from each other at the ends, for here more numerous and enlarged spindle cells occur between the fibrils. The fibers in the bundle are always decreasing in number, retaining their undulating course, and are continued in the coiling fibers of the united interstitial substance.

Mitoses are scarce in the tendon cells. The uniting mass originates from extraordinarily crowded cells with long nuclei, between which fine coiling fibers run in larger quantity. The fibers assume an orderly parallel arrangement, so that ultimately they are not clearly distinguishable from the ones coming from the tendon itself. The fibers on the inner side of the sectioned tendons are maintained, gradually changing into the uniting mass.

Marchand believed the main value in regeneration of the tendon wound lies in the elements of the tendon itself. The tendon sheath and the intertendinous connective tissue take the place of the perichondrium in the healing of cartilage wounds, only with the difference that the elements of the tendon participate more intensively in the new formation than tends to be the case in cartilage.

Vulpius (24) (1902) carried out a series of tendon implantations mostly in the Achilles tendon of rabbits and dogs and was able to confirm healing in, with smooth external results.

The first systematic animal experiments with free autoplastic tendon transplantations were undertaken by Seggel (25) in 1903. He concluded from histologic examination of tendon in guinea pigs that the sheath with the surrounding connective tissue and the tendon participate in the healing process. The space between the cut ends of the tendon becomes filled with blood exudate which is organized by connective tissue from about the stumps and also from the interstitial tissues. By the fifth day mitoses appear on the cut ends and by the sixth day within the substance of the tendon. Proliferating tendon cells infiltrate and gradually replace the nonspecific connective tissue. Continuity is completed by the twenty-ninth day. The tendon

has a normal appearance from the seventieth to the eightieth day.

Borst (26) (1903) agreed with Hoffa in believing that scar filling the defect comes from tendon, internal and external peritenoneum, and the surrounding connective tissue. His experiments with tendons in dogs, cats, rabbits, and frogs included lengthening by Z-sectioning, shortening by a fold in the tendon, suturing one tendon to another, and other procedures. First polymorphonuclear leukocytes, fibroblasts, and then on the fourth day proliferating tendon cells infiltrate the gap. Borst believed that the nature of the operative procedure, the presence of chemical irritants, infection, and suture materials influence the healing process. He gave special study to the tendon nuclei and chromosomes and thought that he was able *to distinguish proliferating tendon cells from connective-tissue cells.*

According to Kirschner (1909) he was the first to make systematic attempts with free tendon transplantations (27). Independently of Rehn, he carried out experiments with free tendon transplantation. The complete Achilles tendon in the dog was replaced by a free tendon transplant on June 14, 1908 (28). In continued experiments the entire Achilles tendon was removed from rabbits and implanted into the muscle gap between the flexors and the thigh in the same animal, without fixation suture. The free transplanted tendon remained partly alive, some tissue being necrotic. He considered the peritenoneum a non-specific tissue which plays a minor role as blood- and lymph-vessel carrier (27). When the Achilles tendon was resected in dogs and the removed tendon piece or a corresponding piece taken from another tendon was placed in the defect and sutured at the ends, the observations were the same. Kirschner concluded that the free transplanted tendon is able to replace the removed tendon functionally (27). He considered the most favorable nutrient conditions to be present when every part of the transplanted tissue can come in contact with surrounding tissue juice. *This condition can be fulfilled if one applies thin tendon grafts* (29) *rather than large thick grafts.*

Homotransplantation of the Achilles tendon with the peritenoneum was carried out in rabbits by Rehn (30) in 1910. On the twenty-first day after operation the transplanted peritenoneum externum had grown to a thick mantle of newly-formed tendon tissue surrounding the transplanted tendon, and nutrition was supplied with the formation of numerous capillary buds. The peritenoneum of the old and the new tendon were united by strong tendon callus, followed by the occurrence of proliferation between the stumps. The Achilles tendon appeared to be thickened. Rehn noted that the tendon transplant showed active regenerative processes. The results of transplanted homotendon with peritenoneum in dogs agreed with those in the rabbit experiments. At the end of four months he regarded the transplanted tendon as a normal Achilles tendon in every respect.

In one series of experiments by Lewis and Davis (31) (1911) pieces were removed from the Achilles tendon of one dog and inserted into a defect in the Achilles tendon of another dog. In another series pieces removed from the Achilles tendon of one dog were inserted into a pocket in the subcutaneous fat of the abdomen of another dog. Transplanted tendons were examined after 7, 17, 21, 35 and 59 days. In all successful experiments the dog could use its leg at the end of a week and could walk without much limp or any dropping of the foot at the end of 14 or 15 days. At the end of 3 weeks the tendon transplant was two to three times thicker than normal tendon. The mantle of tissue is developed partly from peritenoneum externum and partly from subcutaneous tissue forming a bed for the transplant.

The changes in shape and size of the transplants are due to: 1) proliferative changes in the subcutaneous tissue surrounding the transplanted tendon and in the peritenoneum externum and internum; and 2) edema of the transplant, resulting from an imperfect reestablishment of the circulation.

As further described by Lewis and Davis, after 3 to 5 weeks the fibrillae (collagenous fibers) in transplanted tendons seem to be larger than normal, and many seem to be gnarled and twisted, the bundles of fibrillae often being separated by a granular mass. These changes were observed as late as 59 days after operation.

They concluded that the transplant as a whole remains alive, the segment acting as a true viable substitute for the part which had been removed and not as a bridge for developing tenoblasts. Examination of the pieces of Achilles tendon placed in subcutaneous pockets in the abdominal wall at different intervals revealed a tendency of the pieces to become progressively smaller. The fibrillae and nuclei stained well and the piece of transplanted tendon may be regarded as viable. The peritenoneum appeared to play a passive role. Transplanted tendons which assume function early, do much better than those which are immobilized for long periods of time.

Sever (1911, 1912) resected tendons and inserted foreign bodies such as silk, linen thread and catgut in cats. Gross and microscopic observations at varying intervals of 1 week to 2½ years after operation were made. Inserted silk serves as a matrix around which dense fibrous tissue forms. It acts as a guide to regenerative tendinous or fibrous processes (32). Without the presence of the sheath and peritenoneum no true tendon tissue can be regenerated. New tendons, so he concluded, are apt to be larger and stronger than resected ones, especially if silk is used. Provided the sheath and peritenoneum are preserved and function is allowed early, adhesions may not occur (33).

A fragment resected from the Achilles tendon in a dog by Tuvernier (34) (1912) was replaced by a graft from the Achilles tendon of another dog, which had been preserved in a solution of artificial serum at temperature of −1°C. At the end of 2 months the graft was found to be in excellent condition. The continuity of the tendon was perfect. Tuvernier considered the result encouraging for practical surgery.

After excising a piece of tendon attached to various muscles in rabbits, allowing retraction of the tendon ends or simply divided tendons, Henze and Mayer (35) (1914) found that the tendon ends tended to form adhesions. Subsequently, living tissues as a piece of fat from the groin about the tendon ends or a homogenous piece of ear cartilage between the tendon and periosteum, or foreign bodies interposed, all exaggerated the tendency to the formation of adhesions, except the pig's bladder membrane rolled around the tendon as a sheath, which may help slightly. When the tendon was withdrawn from the sheath and substituting tendon was brought through the sheath to its new insertion, or when Lange's silk tendon was used, it constituted a "fascial compartment" and not a true sheath. There was perfect function even after 29 days. Microscopically, the normal space between the tendon and the sheath was preserved. Thus Henze and Meyer concluded that restoration of normal anatomical relations of the transplanted tendon to its environment prevents adhesions. The tendon itself after operation shows extensive necrosis, from which it recovers in 5 to 6 weeks. The dense fibrous tissue enveloping silk strands develops essentially from the adjacent connective tissue though true tendon cells and cells of the peritenoneum and tendon sheath also contribute to its formation.

In a joint publication by Biesalski and Mayer (36) in 1916 it is related how the tendons of the extensor digitorum longus muscle with their sheaths and paratenon in dogs were substituted for the tibialis anticus and immediately used. Two months later the tendons were removed and showed that the four tendon points were grown together in a single tendon, which within its transplanted gliding apparatus moved like an untransplanted tendon and showed almost normal structure macroscopically and microscopically.

In substitution of the sheath by a sheath it was demonstrated where no mesotenon was present that the sheaths through which new tendons were drawn were completely normal. If the tendons had a mesotenon and if this was torn in drawing the tendon out of its sheath, there were tender strands of connective tissue in the lumen of the sheath between the tendon and sheath wall; they offered no hindrance to free sliding. In other places the lumen was completely free. If the mesotenon was not torn out but tied off and separated by the newer physiological technique there were also these tender flexible strands of connective tissue.

In experiments on rabbits in which the tibialis anticus was drawn through the sheath of the peronaeus longus, Henze and Mayer noted the sound appearance and function of the sheath and tendon 15 days after the operation (36).

Mayer (37) (1916) expressed the belief that a rational system of tendon transplantation must be based on an accurate knowledge of the anatomy and physiology of tendons and muscles. He undertook thorough and comprehensive studies at the Oskar-Helene Home for Crippled Children in Berlin and formulated a method of tendon transplantation. This took into consideration the course and insertion of the tendon, the blood supply of the tendon, its fascial relations at various levels, its length, its range of motion, its action, the exact location and inner architecture of its sheath, the character and line of insertion of the mesotenon, and the bursae associated with the tendon. In his opinion, a physiological tendon operation must conform to certain fundamentals: It must wherever possible restore the normal relationship between the tendon and the original sheath; the course of the tendon from its original site to that of the paralyzed tendon must run through tissue adapted to the gliding of the tendon; the normal insertion of the tendon must be imitated wherever possible by implanting the living transplanted tendon directly into bone or cartilage, preferably at the insertion of the paralyzed tendon; the normal tension of the transplanted tendon must be reestablished and the physiological length of the transplanted muscle thus maintained; and the line of traction of the transplanted tendon must be such as to enable it to do the work of the paralyzed tendon effectively.

In Mayer's experiments on dogs, the tendon of the extensor longus digitorum was drawn through the sheath of the tibialis anticus and fastened to its point of insertion. After 4 days physiological union between the tendon and bone had not yet occurred. In rabbits the divided extensor longus digitorum was sutured by the Lange stitch, leaving a gap. On the twentieth day the suture had torn out of the distal tendon stump. He concluded that when tendon is properly anchored, overlapping of mechanical and physiological fixation actually occurs. Furthermore, immobilization of the tendon tends to its degeneration. Muscle also degenerates subsequent to operation if it is not allowed to function. After operation delicate connective-tissue strands form between the tendon and sheath of paralyzed tendon. Loose fatty tissue investing the tendon shows only slight changes when transplanted with the tendon. Here and

there the fat cells are replaced by peculiar cells, the size of white blood corpuscle.

From observations on humans and the reported results of numerous animal experiments, Bier (38) (1917) held the conviction that quick new formation of tendons without a sheath in experimental animals would occur if one does not introduce anything at all, not even a thread between the ends. One should accept that every tissue grows best under natural surrounding conditions. He concluded that not only the maintenance of the gaps is decisive for regeneration but that appropriate nutrient media are required.

Nageotte and Sencert (39) (1918) filled the defect produced by resection of the extensor tendon of the big toe of the paw in a dog, with a graft of dead homogenous tendon. The grafts had been taken a month previously from another dog, placed in alcohol at 90°C. and preserved in alcohol at 50°C. The dead graft was fixed to the two surfaces of the living tendon. Reunion of the wound by primary intention occurred. The dog had no trouble in walking and at the end of some days the appearance of the paw was undistinguishable from its mate. In three months the operated tendon did not differ in any way from the opposite paw, having all the same morphological and physiological qualities macroscopically. The graft of dead tendon formed a constituent part of the living tendon. It was impossible to know where the tendon ceased and the graft began.

Microscopic sections revealed no trace of resorption; the graft was surrounded by a rather thick layer of migrating cells, among which were a certain number of polymorphonuclears. In 20 days, under the same conditions, the connective layer remained intact but the dead cells had been removed by phagocytosis. The vascular rete was reconstituted. Nageotte and Sencert concluded that the connective substance of the graft, which constitutes the essential element of the tendinous graft, had persisted.[2] It is this reconstituted substance that "we have under our eyes"; it is actually living and it is the dead graft itself which has been returned to life.

Rehn (40) (1919) demonstrated in animal experiments that a tendon graft without function underwent retrogressive changes and practically disappeared. If the graft was placed in locations where it was under tension, it remained viable, became attached in its location and replaced the lost tissue. Preservation of the external peritenoneum is essential to success in the grafting of tendons, in Rehn's opinion. He believed that the external peritenoneum makes vascular connections with surrounding tissues and maintains viability of the graft. He concluded that when non-specific tissue was grafted in tendon defects and in other locations of pull and strain, there was metaplasia of connective tissue to tendon tissue of equal value. He further noted that even if no functional stimulus was present, immobilized muscles under a cast were still active; they showed movements which acted as a functional stimulus on the graft.

In experiments in which the Achilles tendon or the largest part of it was excised in dogs, Salomon (41) (1920, 1924) studied the repair process and suture of tendon within a sheath. He ascribed the poor healing ability to inhibition of growth by the synovial fluid, probably through hormonal action, and paucity of tissue capable of proliferation. Tendon healing takes place essentially by proliferation of the peritenoneum externum and internum. When the tendon is not enclosed in a sheath, the paratenon provides abundant tissue capable of

[2] Connective substance probably means the collagenous fiber bundles of the graft and not the tendon or connective-tissue cells, which were dead at the time of transfer. Any living cells would have to be infiltrating cells from the host tissue.

proliferating. Union would occur, however, if tenotomy was done above the sheath and the tendon was sutured within the sheath, the stumps being thus held together. He suggested that the sheath be left open or part be excised in intravaginal suture so that the suture line come in contact with the subcutaneous tissues.

In Gallie and Le Mesurier's experimental series on rabbits in 1921, section of the tendo Achillis, or one of its component parts, was cut free from its circulation and then sutured back in place as a free graft with black silk thread. Specimens were examined at varying intervals up to 13 months. The cells and fibers continued to live and underwent only the change produced by inflammatory edema. No absorption, invasion or infiltration occurred, and there was no evidence of proliferation of the essential cells. They noted that if the transplanted tendon is thick, the central portions degenerate owing to the absence of an adequate supply of lymph. In the healing process, therefore, "*the connective tissue which forms at the point of union arises from cells which are not tendon cells, and which cannot be expected to reproduce true tendinous tissue.*" These cells form an ordinary scar. The scar tissue fibers, being very irregularly and loosely arranged, show a constant tendency to stretch. Gallie and Le Mesurier came to the conclusion that before the transplantation of tendinous tissues could become of real clinical value, a method must be devised to secure a firm union of the transplant to the surrounding structures (42).

In another study of free transplantation Gallie and Le Mesurier (43) (1922) found that healing of tendon to bone occurs by the formation of new connective tendon, which adheres to both tendon and bone. If the tendon is split so that its raw surface comes in contact with bone over a sufficient surface area, the fixation will withstand any degree of physiological strain. They believed that if fascia, tendon or aponeurosis is transplanted in the same animal, the tissue as such will continue to live unchanged.

Working with rabbits, Schwarz (44) (1922) noted the same results as many earlier investigators, i.e., the new formation of tendon resulting from the external peritenoneum, and only slightly from the internal peritenoneum, while the tendon tissue itself is passive. If sufficient external connective-tissue sheath is maintained, then a new tendon is formed. In another series the external peritenoneum was considered as having no specific tendon-forming properties.

Schwarz also removed the external peritenoneum and the remaining connective tissue in its neighborhood before transplantation. A part of both Achilles tendons of the rabbit were resected with the peritenoneum, and a corresponding part of the right side transplanted into the defect and brought in contact with the stumps. Sections were examined macroscopically and microscopically at intervals of 5 to 123 days. In all experiments after transplantation substitute tendon developed, which was thicker than a normal Achilles tendon of the rabbit. Schwarz perceived as certain that the main part of the new tendon came from proliferating processes of the transplanted peritenoneum. He was convinced that the proliferating processes of the transplanted peritenoneum and the formation of substitute tendon are under the influence of and in causative relation with the functional stress. Some cases reported confirmed what he found experimentally. He considered it hardly profitable to transplant tendon tissue in tendon defects.

Wehner (45) (1923) excised the patella in dogs and sutured the patellar tendon over the open knee joint where the suture line was constantly bathed in synovial fluid. The tendon was observed as having regenerated perfectly.

In experiments on dogs, Hueck (46)

(1923) sutured the flexor tendons of the toes in the ball of the foot where they are contained in synovial sheaths. In some instances the sheath was carefully closed over the tendon suture; while in others it was left open or cut away in part. But when apposition had been good, the stumps soon separated and were covered by a very small cap of tissue. Whether the sheath was open or carefully closed, failure was apparently caused by inability of the connective tissue to proliferate.

In the experimental work by Weidenreich (47) of Heidelberg (1924) pieces of flexor tendon were removed from both heels of the dog, preserved for several days and then transplanted into the flexor tendon of another dog. After 8 weeks there was healing in, and stained sections gave the impression of normal tendon tissue. Weidenreich considered that the preserved tendon graft had lost its original cells in the host body. New cells, true fibroblasts, had migrated from the surrounding connective tissue of the host, and fiber bundles resembled the original ones in number and location. He concluded that there was no actual healing in, neither in the sense of complete maintenance of the transplanted tissue nor in further utilization after change in the cells. Sooner or later the transplant disintegrates and is replaced to the same extent from its surroundings.

In experiments on rabbits with daily injections of vital staining Imayoshi (48) (1925) noted that the healing of the defect in tendon is due to tendon-cell proliferation. The histological observation was normal vital stained rabbit tendon. He believed that by this method he had been able to distinguish between tenoblasts and fibroblasts. The first tissues to fill up the gap are fibroblasts, which are later replaced by tendon cells, or tenoblasts from the stumps.

In a series of experiments on dogs, Garlock (49) (1927) divided tendon transversely after separation of the mesotenon from the posterior aspect, and sutured it with fine silk in the proximal and distal stumps. The repaired tendon was adherent to the tendon sheath at the site of the suture when examined on the ninth, eleventh and twenty-sixth day after operation. In another series a portion of the tendon was excised from the left hind leg, and a free tendon graft taken from the right hind leg was inserted into the deficiency; the parts were immobilized. Garlock concluded that the repair proceeds along definite lines. Free tendon grafts inserted to bridge a defect in a tendon live as such. "The return of function following a tenorrhaphy, or the insertion of a free tendon graft, is dependent upon an intact suture line, a return of its muscle belly to a normal state, and the breaking away of the tendon from its surrounding tendon sheath."

Lange (50) noted experimentally that the regenerative power of the tendon itself is not great. If the external peritenoneum and the subcutaneous tissues were not allowed to enter into the formation of tendon callus, regeneration of the tendon did not occur.

After experimental and clinical study of the relation of tendon to connective tissue in the healing of a tendon, Närvi (51) (1931) held the view that the ability of tendon to regenerate is weak and that tendon granulation develops from connective tissue surrounding the tendon stumps. The Achilles tendon of a cat showed no great regenerative reaction 6 weeks after resection. In further research, he concluded that the healing of a defect in tendon is due not to regeneration issuing from the ends of the divided tendon but from surrounding connective tissue.

In the study on the process of tendon repair in dogs, by Mason and Shearon (1) (1932) first a tendon was divided and immediately sutured. In the same series a segment was excised from the tendon and the gap was replaced by a tendon graft taken from

the corresponding tendon on the opposite leg; in other instances the tendon removed was replaced in the gap to serve as a graft after it had been completely severed from its blood supply. In some experiments the divided sheath was sutured over the graft; in the later experiments, however, sheath suture was not done. The course of events in the specimen sections was studied microscopically at verying periods from 4 days to as long as 100 days in one instance.

To quote, "although the basic histologic process is the same in both the simple end-to-end suture and in the graft, the presence of a tendon graft in the defect is seen to play a definite role in healing."

During the first phase of the healing process, lasting for 2 weeks, the union between the graft and the stumps is brought about by proliferation of the sheaths. In the second phase, overlapping the first, at about the fourth or fifth day after operation, the tenoblasts proliferate, bridging the separation of the tendon and the graft. At this time the first mitoses are apparent. From the second week on, new tendon is formed essentially from the organization of a scar lying between the ends of the graft and the stumps, the tenoblasts playing the most important part. As the role of the sheath as a uniting structure diminishes, it begins to assume its gliding function. During the fourth and fifth postoperative weeks, the sheath becomes more easily separated from the organizing tendon, and when its union is attained, a tendon with a paratenon around it is well organized.

They explain the significance of this healing process in a tendon graft. Based on these experiments the conclusion is drawn that tendon with its surrounding connective tissue maintains its vitality when transplanted as a free graft. The sheath first fuses with the sheath tissues of the host tendon, and the tendon cells in the graft and in the host tendon proliferate and join the tendon graft to the host tendon.

In 1932 Hesse (52) summarized the different conceptions of the healing of tendon defects as found in the literature. The healing process can be effected 1) through formation of a specific tendon tissue from tendon cells; 2) through formation of a connective-tissue substitution tissue, proliferated from cells of the peritenoneum and spongy connective tissue enveloping the tendon; and 3) through scar formation. In Hesse's experiments on dogs and rabbits, reconstruction occurred more quickly in free autogenous transplants of tendon without a sheath than in one with a sheath when implanted in the knee joint. The specific, highly-differentiated tendon elements were destroyed but connective tissue belonging to the tendon was involved in the reconstruction of the tendon in association with the recipient tissue. The free autogenous pieces of Achilles tendon and the tendon of the flexor hallucis implanted in the knee joint of dogs under tension maintained their structure, the fibrils being arranged lengthwise. The staining of the cells and fibrils in areas other than the severed places in the middle of the transplant is uniform. The autogenous tendon transplants in the synovial cavity without functional stress lose their specific character and behave as if they were displaced subcutaneously. The reconstruction of the transplant succeeds in sheathless tendons rich in connective tissue more quickly than in synovial ensheathed tendons. Hesse considered the tendon sheath and the joint cavity morphologically to be much alike.

In experimental work, Kernwein and his associates (53) (1938) transplanted [presumably autogenous] tendon, fascia lata, or white connective tissue, and ligamentum nuchae into the defect of the tibia in rabbits or of the femur in dogs, which were sacrificed at intervals of from 60 to 391 days. The ligamentum nuchae, tendon, and fascia lata

of both species suffered nutritional disturbances but remained viable and tended slowly to become ossified. Ossification of the transplant was observed to be most marked in the cortical area and least in the medullary portion. Ossification of the transplanted soft tissue occurred in two ways: 1) by replacement by invading osteoblasts which formed bone; and 2) by metaplasia. Kernwein believed that the firm anchorage obtained by passing tendon through holes in bone is due to their gradual ossification and incorporation in the bone. He found that lack of function had no demonstrable effect on the changes he had observed.

Writing in 1941, Mason (54) stated that there is no real agreement on the actual nature of the repair process in tendon, whether union occurs by scar tissue formation or by the activity of specific connective-tissue cells or tendon itself. Histologically there are two stages: one corresponding to the initial healing of any tissue, namely, the formation of connective-tissue scar, and the other, specific healing.

The flexor carpi ulnaris tendon in dogs, which had been separated from its mesotenon, was cut transversely and sutured. Examinations were made at varying intervals. During the first phase of healing there was rapid diminution in strength and during the second phase there was an increase in strength up to about the sixteenth day. Function has an accelerating effect on tensile strength, which starts on the nineteenth day and continues for an indefinite period.

In experiments on the extensor carpi radialis tendon and the flexor carpi ulnaris tendon in dogs, as reported by Mason and Allen (55) (1941), the sheath was split open; then tendon was separated from its mesotenon, divided transversely and sutured with silk in a manner to change the line of pull of the suture from a longitudinal to a transverse direction. This procedure left free the tendon ends which were to take part in the healing process. The sheath was not closed. The sutured tendon was examined and removed at varying intervals of 2 to 68 days. Tendon healing as measured by its tensile strength shows three phases: a phase of rapid diminution (about 5 days), a phase of increase in tensile strength up to a plateau (about the sixteenth day); and another phase of increase in tensile strength (from the nineteenth to the twenty-first day). Curves of tensile strength conform to phases observed in the histologic process of repair; a phase of exudation and fibrinous union, a phase of fibroplasia, and another of maturation or organizing differentiation. The function to which tendon is subjected is reflected in the curve of the third phase of healing. Function and motion during the first two phases of healing lead to increased reaction and to separation at the suture line.

In Kernwein's experiments reported in 1942, the tendon of the extensor carpi radialis longus muscle was transplanted satisfactorily into both radii, presumably as autografts, in dogs. In one group the tendon, with its sheath retained, was fitted into holes of varying size.

In another group, a portion of tendon without its sheath was implanted in a bony tunnel, its component fibers being separated and bone sand from the drill-hole being interposed among the strands. The animals were sacrificed at intervals of 1 to 173 days and the tensile strength of the union between the bone and tendon was measured. Kernwein believed that firm anchorage from tendon drawn through a drill hole is due to the ossification and gradual incorporation of the tendon in bone. During the lag period of union the tensile strength falls below that of the initial mechanical fixation. The relative size of the tendon to the drill hole has little effect upon the rate of union between the tendon and bone. Bone sand placed between the strands of shredded tendon shows aseptic necrosis and is resorbed. He

also concluded that shredding of the portion of the tendon implanted in bone permits the surrounding fibroblasts to permeate, and by increasing the surface area of anchorage, accelerates the rate of the rise of tensile strength (56).

In a study by Buck and Wilhelm (57) (1952) cortisone acetate was given intramuscularly to albino rats, and the operative procedure was carried out 48 hours after the first injections. The cortisone injections were continued until the animals were sacrificed. The right Achilles tendon of the cortisone-treated rats was transected, without suture of the severed ends and without immobilization. The rats were sacrificed at 5, 10, 14 and 21 days after operation. The endotracheal curettage was performed on the anterior surface of the trachea in cortisone-treated rats; the animals were killed at intervals of 4 hours to 14 days, and examinations made. The administration of cortisone results in the production of a substitute tendon histologically similar to the regenerated tendon of untreated animals but less in amount. Cortisone does not alter the rate of epithelization of shallow tracheal wounds produced by curettage.

Skoog and Perssen (58) (1954) made a study of the parts played by endotenon, peritenon, and paratenon in the healing of tendon. They precisely define *endotenon* as the connective tissue between the bundles of a tendon; *peritenon*, as the connective tissue covering the surface of a tendon; and *paratenon* as the connective tissue outside the peritenon and not belonging to the tendon structure proper.

In one group of rabbits the Achilles tendon was simply divided transversely, the sheath was scraped off the tendon previously, or the cut ends of the tendon were enclosed in thin stainless steel foil. In a second group the experiments were repeated except that the divided tendon was sutured. In a third group the segment removed from the tendon was replaced and resutured as a free tendon graft. The conclusions drawn from macroscopic and microscopic examination of specimens at intervals of 4 to 21 days were as follows: Scar tissue which forms between the cut ends of the tendon arises mainly from paratenon; tendon itself does not appear to make any contribution. Peritenon alone is inadequate to permit healing within a normal period and endotenon plays no part in regeneration. If the peritenon is left intact, however, the reaction is less violent in the surrounding tissue. In simple division of the tendon the peritendinous connective tissue performs no significant regenerative function during the first 3 weeks.

If the peritenon is scraped off the isolated tendon, the endotenon plays no part in tendon healing; there is a great increase in the surrounding connective tissue. Suturing of the cut tendon does not appear to affect the healing process. A free autogenous tendon graft seems to be replaced by new connective tissue, which grew in along its entire surface, being controlled by the collagenous fibrillary bundles of the graft. The endotenon does not appear to play any active part in the regenerative process in tendon grafts, and no specific rôle can be assigned to the peritenon. *Skoog and Perssen concluded that the cells in free autogenous tendon grafts degenerate, and that the graft itself is replaced by new connective tissue, which grows in along its entire surface.*[3]

The screening of the lesion from the surrounding tissues by a thin tube of stainless steel foil led to considerable delay in healing.

The paratenon was the main source of the new connective tissue which arose in the gap between the cut ends of the tendon, or in

[3] This observation on autogenous tendon grafts in rabbits is similar to the creeping substitution theory in bone grafts (absorption and replacement of a bone graft in contact with bone) excepting that the replacement occurs from infiltrating host cells in the surrounding connective tissue and not from the host tendon.

the healing and replacement of free autogenous tendon grafts. The host tendon does not participate in the replacement of the tendon graft (58).

SUMMARY COMMENT ON AUTOGENOUS AND HOMOGENOUS TENDON GRAFTS IN ANIMALS

The process of tendon repair has been studied for centuries relative to the healing of severed tendons, and later the healing of free tendon grafts, and the survival, absorption or replacement of the graft structure.

Tendon grafts in contact with host tendon are suggestive of bone grafts in contact with host bone, so there is a temptation to predicate a creeping substitution theory for the grafted tendon. Or, taking a middle ground, one may suppose that the cells in autogenous tendon grafts survive like the cells in autogenous cancellous bone grafts, and that the tendon becomes joined to the host tendon by fibrous union, as the cancellous bone graft becomes joined to its host bone by bony union. Fresh and preserved homogenous tendon grafts may be replaced by creeping substitution from host tendon or from the surrounding host connective tissue.

There is still much controversy, however, about the precise nature of the healing process in tendon repair and the survival or replacement of autogenous tendon grafts.

Skoog and Perssen reviewed the conflicting opinions as follows: One view is that regeneration takes place by outward growth from the cut ends of the tendon (v. Ammon, 1837; Garlock, 1927). Seggel (1903) performed some experiments on guinea pigs which showed that the space between the cut ends became filled with blood that was later organized by fibrous growth from the internal and external tendon sheaths. This non-specific connective tissue was subsequently replaced by specific tissue from the tendon itself. Similarly, Mason and Shearon reached the conclusion that union is first affected by proliferation of the tendon sheath tissue (epitenon), but that the tendon itself begins to proliferate in dogs after the fourth or fifth day. This conception of the process of repair was supported in experiments on dogs by Mason and Allen (1941). These investigators also noted that *the tendon cells in free autogenous tendon grafts survive transplantation as living cells and participate in the repair process.* The microphotographs supporting these conclusions are just as impressive as those supporting the opposing conclusions of Skoog and Perssen, which were reported recently. Imoyashi in 1925 had also noted that healing in tendon defects in rabbits is due to tendon cell proliferation.

Another school of thought holds that new tendon arises mainly from the sheaths that surround the tendon stumps, and that the tendon tissue itself plays no significant role (Adams, 1860; Hueck, 1924; Närvi, 1931). Marchand (1901) believed that most of the new tissue came from the sheath and surrounding connective tissue which proliferated into the ends of the tendon stumps, but he also admitted that it might be impossible to distinguish between mitotic tendon cells and connective-tissue cells. Gallie and Le Mesurier (1921) concluded that healing did not occur through the activity of tendon cells in the graft or host tendon *but the tenoblasts in autogenous grafts survived as such and were not replaced by host tissue cells.* Schwarz (1922) never observed tendon cells actually taking part in the formation of new tendon tissue.

Skoog and Perssen (1954), in careful experimental work with rabbits, concluded that repair occurred mainly from connective-tissue cells in the paratenon, and that tendon cells or connective-tissue cells in the endotenon did not participate in the process. The peritenon covering the tendon is essential to a good functional result in tendon suture and free tendon grafts, because fewer

fibrous adhesions occur when peritenon is present between the tendon and surrounding host connective tissue (paratenon).

When the peritenon[4] is present, it is possible for the surrounding tissues (paratenon) to revascularize the tendon by blood vessel anastomoses with the original vascular pattern of the tendon (59), and the peritenon also provides for some gliding mechanism.

According to Skoog and Perssen, the peritenon is not vital to the repair of the tendon after injury but its presence is essential for normal function. Surprisingly, they noted that *the cells in a free autogenous tendon graft degenerate after transplantation, and that the graft is replaced by infiltrating cells from the surrounding host connective tissue.*

A possible explanation for the different findings may be the fact that different experimental animal species were used (rabbits and dogs). I have buried free autogenous human tendon grafts in abdominal fat and noted after removal 10 months later that the tendon had a normal gross and microscopic structure. Sections removed at 1 to 6 days, 1, 2 and 4 weeks did not show evidence of absorption and replacement from the surrounding host connective tissue or degeneration and death of the tendon cells. The tendon was transplanted with its slippery paratenon (peritenon) covering. One is inclined to believe by inference, therefore, that the tendon cells in autografts survive as such in the human and possibly also in the dog. It is interesting to note that Imayashi, using vital stains, decided that the tendon cells take part in the healing process (in rabbits), whereas Skoog and Perssen, also working with rabbits, were unable to confirm this observation.

These conflicting opinions based on factual evidence regarding the fate of autogenous tendon grafts in animals are difficult to evaluate. It is possible, of course, that tendon grafts react differently in the different species experimented upon but, alternately, experimenters observed different findings when they buried tendon grafts in the same species.

Admittedly, it is very difficult on the basis of fixed and stained sections to differentiate the fibroblasts in the stroma between tendon bundles, and the tendon cells from infiltrating host fibroblast cells, which are assumed to enter the graft structure (if this ever occurs). The regularly-arranged tendon cells may also undergo change in a free tendon graft so that they cannot be readily differentiated from fibroblast cells in the stroma of tendon, or from infiltrating fibroblasts from the surrounding host connective tissue.

The gross and microscopic appearance of autogenous tendon grafts buried for long periods of time in animals indicate rather definitely that *the graft area is occupied by tissue which is the exact duplicate of normal tendon.* Authorities differ, however, as to whether this "normal-appearing tendon" represents the tendon graft as such or is actually a counterfeit substitute produced by infiltrating fibroblasts from the host tissue, which replaced the original tendon cells in the graft. Experimenters do not commit themselves regarding the survival of the collagenous and elastic fibers in free autogenous tendon grafts in animals, the blood vessels, and their endothelial cells, or the survival or death of the fibroblast cells in the connective-tissue stroma between the tendon bundles.

The evidence concerning the fate of fresh and preserved homogenous tendon grafts is still more obscure. Good clinical results are reported by some investigators, with evidence that the graft structure has been replaced by the host tendon or surrounding host connective tissue. The replacement

[4] Skoog and Perssen use peritenon to denote the slippery gliding tissue covering the tendon; Bunnell and others call this paratenon. Skoog and Perssen use paratenon to designate the host connective tissue outside the slippery covering.

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SUMMARY COMMENT ON AUTOGENOUS AND HOMOGENOUS TENDON GRAFTS IN ANIMALS

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lagen fibrils without cement substance or cellular parts. Implants were made in adult guinea pigs intramuscularly, under the abdominal skin, and in spongiose connective tissue of the inguinal region. After 3 days there was no union of surrounding tissue with the implant. Bundles of fibrils were present around the middle of the implant, as was a layer of cement substance. At the margins were reticular fibers and an accumulation of cells. After 9 days the fibrils increased, more cells penetrated from the interstitial spaces and from the margins, whereas the number at the center diminished. Passage of implant fibrils into the surrounding connective tissue was observed; the formation of a fibrous capsule stood out. In 21 days the immigration of cells was abundant. The thick packing of fibrils of the original substance was no longer observed anywhere. In some places the fibrous capsule was united with the fibrils. A large number of capillaries were seen within the area of the implant. In summary, after the initial infiltration of the implant with tissue serum, there followed a laying down of substances which caused an agglutination of fibrils not visible under the microscope. By electron microscopic examination reconstituted collagen fibrils formed an impenetrable fine network and very little cement-like substance was observed. It is Bahr's impression from the present stage of his investigation that the implant broke down in the main and was replaced by new material coming from local tissue.

SUMMARY COMMENT ON HETEROGENOUS TENDON GRAFTS

From the small number of investigations of heterogenous tendon grafts in animals reported in the literature, one is inclined to believe that such tendon grafts are inferior to homogenous tendon grafts. These, in turn, are not as good a grafting material as fresh autogenous grafts. The cells in fresh heterogenous grafts die after transfer and the cells in preserved heterogenous grafts are already dead. The collagenous bundles in both instances are probably absorbed over a period of time. There is some effort at replacement by host tissues, and host cellular reaction is more intense than that in homografts. The precise ultimate fate of heterografts of tendon in animals has not been determined at this time. The experiments by Bahr in 1952, however, indicate that the collagen fibers in heterogenous grafts are absorbed and replaced by new collagen fibers through the activity of host fibroblasts. There are no reliable data regarding the microscopic appearance of the replacement tissue in heterogenous grafts buried for long periods of time so that it might be compared structurally with normal tendon.

As physicians we are interested in animal experimental work because it helps us to determine what happens to similar grafts in humans. We must remember, however, that veterinarian surgery has developed rapidly in recent years, and the time may come when the behavior of tissue grafts in animals will be applied clinically among species which are useful to man. In the future, veterinary surgeons may be interested in the behavior of human tissue grafts in order to determine the value of similar transplants in different animal species.

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structure is in the form of connective tissue but does not have the specific structure of a true or normal tendon in the opinion of some, whereas the replacement structure is an exact duplicate of normal tendon in the opinion of others.

A number of experimenters believe that the cells in homogenous grafts are replaced by infiltrating host tissue cells or by cells from the host tendon or its paratenon covering. Some early investigators stated that the cells in the fresh homografts survive but this seems improbable. No definite conclusions have been reached in regard to the fate of the collagenous and elastic fibers in homogenous tendon grafts.

The source of the cells taking part in the healing of homogenous tendon grafts at points of contact with host tendon is not known, since the various investigators disagree. Some believe that healing occurs through the activity of host fibroblasts or undifferentiated connective-tissue cells, whereas others feel that host tendon and paratenon cells participate in the process of repair.

HETEROGENOUS TENDON GRAFTS

Fargin and Assaky (60) in 1885 transplanted pieces of tendon from the dog and ram into rabbits, and similar pieces of tissue from rabbits into dog, and from birds into rabbits, and *vice versa*. The results were reported as being equally favorable.

Vulpius (61) in 1902 reported that he carried out a series of implantations similar to those by Fargin and Assaky, mostly in the Achilles tendon in rabbits and dogs. He was able to confirm the smooth healing in of the transplant.

After these rare early attempts at transferring tendon grafts from one animal species to another, any worth-while reports are scattered indeed, appearing after a long lapse of years.

Horsley (62) (1931) tied autogenous fascial transplant from the rectus sheath, alcohol-preserved fascia lata of the ox, and chromicized kangaroo tendon snugly about the pylorus, and loosely about the small bowel in the peritoneal cavity, and in the abdominal wall of dogs, the experiments extending from 5 days to a little more than 6 months. Observations were also made on humans. The kangaroo tendon soon became encapsulated and was absorbed more quickly in the abdominal wall than in the peritoneal cavity. Chromicized kangaroo tendon was much more satisfactory for pyloric occlusion in the dog than fascia, forming total occlusion for more than 6 months and causing fewer adhesions than fascia. The tendon also showed a high degree of resistance to absorption and infection.

When grafts of dead tendon were inserted under the skin of the rabbit's ear, Nageotte (63) (1939) noted that after remaining in place for 4 months they were preserved as an integral in the form, structure and appearance which they had had at the time of operation. He made these conclusive observations: A fragment of heteroplastic tendon in which all the living elements have been killed by fixation never behaves like a foreign body in living tissues into which it has been inserted, provided no infection is present. The connective stroma of the dead tendon graft is soon united to the stroma of the host tissues. This process of union of the stroma of dead tissue with that of living host tissue is identical to that by which two lips of a wound made in a living tissue are reunited by primary intention. The fibroblasts from the host tissue are mobilized around the dead graft, which they envelope and replenish. Revascularization of the dead graft is by invasion of the proliferating host vessels. Nageotte believed that thus the structure again becomes viable.

Bahr (64) (1952) experimentally put fine fibril material from rat-tail tendon through a dissolving process in order to obtain col-

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by Schwartz (7) of Buffalo, in 1847. At the suggestion of a friend of the patient the wound on the anterior carpal surface exposing the common flexor tendons of the fingers was covered with soap and sugar. The tendons became disorganized and broken by slough. Though not allowed to treat the wound Schwartz at intervals did observe the progress and result. A union of the superficial tissues occurred by granulation and the mobility of the fingers was perfectly restored.

Adams (8) in 1860 noted that the line of junction of the old with the new tendon could be recognized in the human tendon 3 years after division. Old tendon retains its opaque, dense, glistening white character, while new tendon possesses a certain amount of translucency and is of a uniformly grayish color, without the characteristic glistening appearance of the old tendon.

Malgaigni (9) (1862) agreed with Velpeau in advocating the suturing of severed tendons to adjacent tendons.

In a case of open phalangeal joint and severed tendon of the extensor longus pollicis muscle, Parsons (10) (1871) placed the thumb in a gutta-percha splint and at perfect rest. The tendon evidently became united, for the patient later was able to extend as well as flex the thumb in a manner prior to injury.

Operation for the relief of deformity seems to have originated with Nicoladoni (11) of Innsbruck in 1881. He advocated sewing the transplanted tendon to the paralyzed tendon. In calcaneus paralyticus (P. calcaneus s. strictiori) with characteristic deformity of the sole he transferred the tendons of the peronei muscles to the bisected Achilles tendon, with a good result.

It is to Czerny (1882) and Bouglé (12) that Rehn and also Marchand attributed the first attempt to carry out an autoplasty with tendon in a human. The tendon material was taken from the same patient and applied to replace a small tendon defect, with a satisfactory result.

Volkmann (13) (1882) and Witzel (14) (1887) made known the kind of surgery undertaken and their results in the healing processes in tendon wounds.

In a patient in whom the extensor tendon of the index finger had been completely torn away, Robson (15) (1888) made a successful restoration with a whole tendon graft, "the first time, to my knowledge, this had ever been done." He removed from its sheath 4½ inches of the flexor tendon in an irreparably injured finger and grafted it on the dorsum of the hand so as to form a new extensor for the index finger. Subsequently the patient was able to perform his duties as a weaver as efficiently as ever and was satisfied with his limb. The hand had a curious shape (weird one might say) but very good movement.

Lange (16) (1892) described lengthening of quadriceps tendon in an old fracture of the patella by cutting across the tendinous portion within its connection with the lateral muscular tissue, which permits considerable stretching. He pointed out that continuity of the organ was preserved by this procedure while lengthening of the tendon was achieved.

Drobnik (1896) proposed the implantation of a tendon graft in the bone at the insertion of a paralyzed tendon which was too short. He was said to be the first to employ an operation for paralysis of the upper extremity. He obtained brilliant results in some of 16 operated patients (17). Kirsch (1897) reported a case in which he buttonholed the peripheral ends of the cut extensors of the thumb into the extensor carpi radialis longus (18).

Rose and Carless (19) (1898) described the manner of repair in a tendon. As soon as a tendon is divided, the ends retract to a greater or less extent, leaving a space which is at first filled with blood clot. This is changed into vascular granulation tissue and finally into a fibrous cicatricial tissue. In this way the tendon becomes lengthened.

CHAPTER 25

Transplantation of Tendon In Humans

It is surprising to note that as early as the tenth century there was an interest in the operation of tendon suture. One must realize, however, that only a few individuals during subsequent centuries undertook what had been considered a hazardous surgical procedure. Perhaps one of the earlier steps was the venture of Moinichen, who in 1665 published some successful cases of suturing of divided tendons (1). In 1698 La Vauguion (2) advocated the suturing of tendons in long-standing injuries; he excised the cutaneous cicatrix and freshened the ends of the divided tendon which had become "callous." Naturally suturing of severed tendons preceded the grafting of tendons by much experimental work, as well as by many clinical observations.

REVIEW OF LITERATURE ON AUTOGENOUS AND HOMOGENOUS TENDON GRAFTS

It is said that free tendon grafting was possibly first performed by Nisson (3), who in 1770 reported a case in which the tendon of the extensor of the middle finger was severed and could not be sutured. The proximal end was grafted upon the tendon of the index finger, and the distal end implanted into the tendon of the ring finger.

Valentin (4) in 1839 probably had grafting in mind when he reported a case of a large wound of the index finger in which the extensors and communis were sectioned. A single thread drawn through the lower end of the extensor proper, and two threads through the common extensor, were passed across the upper end in such a manner that they "were just like grafts." The wound united by primary intention, resulting in agility in the use of the hand. In a second case threads passed through the thickness of the lower ends of the severed tendons of the first and second radial muscles were brought into approximation. After primary healing flexion of the fingers became easy, associated with movement in the wrist.

Velpeau (5) (1839) recommended that severed tendons be sewed to adjacent intact tendons. He made observations on tendon wounds in patients who had suffered rupture of the Achilles tendon. He believed that the union in tendon took place rapidly like the healing of bone, in a similar cartilage model form. Healing of sectioned tendons occurred by primary intention, the tendon sheath playing the most important rôle. Both Velpeau, and Bouvier (6) (1847) held that the newly-formed fasciculus between the severed tendon ends developed from the thickened tissue of the tendon sheath.

As illustrative of the ability of severed tendon to unite even though neglected surgically and treated only by folk method is the instance cited

of paralysis had become a very gratifying chapter of orthopedic science (26).

A patient who had been treated previously for a deep infection of the hand with invasion of the articulation of the wrist was presented by Dupage (27) (1907). Opposition of the thumb with the other fingers was impossible. The tendon of the flexor profundus of the medius was divided in two; half remained attached to the finger, and the central half was transferred to the thumb and sutured to the base of the last phalanx. Healing took place by primary intention. A month after the operation opposition to the fingers was already possible.

Rehn (28) in 1909 recommended tendon tissue as implants for tendon defects, maintaining that his transplants of tendon showed persistent viability with retention of gliding peritendinal surfaces. This gliding apparatus was regarded by him as essential for the functional success of a transplant in a tendon defect. Since the amount of tendon tissue obtainable for autoplasty was limited, he studied homotransplantation of tendons, and believed the results were quite satisfactory.

Rehn (29) (1910) performed free auto- and homotransplantations of tendons in humans and noted that in homografts the specific tendon-forming property of peritenoneum is essential. In addition, the favorable influence of functional stimulus on the transplanted tendon tissue and the restoration of the original tendon tension are factors to be considered.

Lange (30) of Munich in 1910 lengthened the transplanted tendon with strands of silk impregnated with paraffin. He was not satisfied with the results obtained in about a thousand transplants.

In a case of injury to the lower end of the radius and tissues of the hand reported by Murphy (31) (1912), the thumb became gangrenous. A large flap was freed from the abdominal wall and sutured onto the wrist to fill in the gap, restoring circulation although a small portion of the thenar eminence became necrotic. Two months later a portion of the original graft was swung around to fill in the gap between the ulna and the radius. All flexor tendons had been divided by the injury. The gangrenous tissue of the thumb was removed and the tendons in this area were cut off and left loose. Two months later these loose tendons were caught up and sutured to the fat and fascia of the palm of the hand. One year and a half later, part of the ulna was removed to permit flexion of the wrist and at the same time all of the flexor tendons were elongated and reattached. These tendons were then carried forward through the adipose tissue of the skin graft. The patient had a fairly good use of the hand after the last operation and was able to return to his occupation as a brakeman.

In a case of a child with an upper motor neuron lesion of congenital origin, reported by Murphy, the active flexor carpi ulnaris was united to the flexor sublimis and the profundus digitorum of the ring, middle and little fingers; the flexor carpi radialis, to the flexor pollicis longus and flexor indicis; and the flexor profundus digitorum of the index and little fingers, to the flexor carpi radialis; each of these having their tendons elongated 1½ inches. The operation resulted in the ability to hold the wrist straight out, and motion was slowly returning to the fingers at the time of dismissal.

In a fracture at the junction of the lower and middle thirds of the radius, all the tendons were lengthened, including the palmaris and both the flexor carpi, as well as the flexor tendons. There was immediate primary union. After additional lengthening of the tendons, movements of the hands and fingers became practically normal. Later perfect flexion and extension of all fingers, thumb, and wrist were possible. Murphy re-

This new tissue is at first adherent to the sheath but later it may regain free mobility within its envelope.

Hoffa (20) in 1899 reported on 26 cases of transplantation of tendon in 22 patients, 15 being active, 6 passive, and 1 active-passive transplantation. In a number of these transplantations there were associated tendon shortenings, one, a tendon injury, and one, a tenotomy of the Achilles tendon; in four instances tendon shortening alone was carried out. All grafts healed smoothly; on the whole the results were satisfactory.

Fritz Lange (21) (1900) recommended that the cut ends of active tendon be sewed to the periosteum, as this is the normal attachment.

One of the first to transplant fibrous tissue successfully for support in hernia was McArthur (22) (1901). He demonstrated the feasibility of using the patient's own living tissues as the means of preventing secondary hernia. A strip of tendinous tissue of the external oblique muscle may serve as the living suture. Its application avoids the introduction of dead or foreign tissue, thus diminishing the chance of failure. Organized white fibrous tissue becomes incorporated in the cicatrix. McArthur tried utilizing this method in 20 cases, in all of which there was perfect primary union.

Similar experiments with the aponeurosis of the external oblique muscle in the dog demonstrated that the transplanted tissue heals in place, is not absorbed, and does not slough.

In two cases reported by Mainzer (23) (1902) direct homotransplants of tendon were used successfully to bridge defects. In the one case a free tendon graft from the paralyzed peroneus tertius was used as a direct transplant to bridge a space between a flap taken from the Achilles tendon and the extensor longus digitorum. The patient could walk normally after a year. In the second case tendon of the peroneus tertius was used as a free transplant to unite a flap from the Achilles tendon with the extensor longus digitorum. The dorsal flexion of the foot was good after 7 days.

Fritz Lange of Munich, then called the master of tendon surgery, used silk sutures if the tendon of the transplanted muscle was too short or too thin, in order to obtain more assured results in his method of suturing the split-off tendon directly to the periosteum. In all instances he lengthened the tendon with silk and noted primary healing. No suture abscess was observed after the first operation 2½ years previously. The functional results were satisfactory. Lange (1902) believed that the durability of the result is guaranteed by the fact that a tendon of real tendon tissue forms around the silk suture (24).

Krause (25) independently conceived of and carried out a periosteal method of transplantation of the flexors on the front of the knee joint. He fastened the tendon on the upper border of the patella.

In addition to working with animals, Vulpius of Heidelburg had an extensive experience with tendon operations in humans. In a monograph published in 1902 he gave a large number of surgical procedures for tendon transplantation, especially for paralysis of the foot. He believed that the anatomical restitution does not keep pace with favorable functional results. In the healing process young tendon tissue creeps along silk threads as along a lattice. The artificial tendon serves as a guide to the young tendon. The silk tendon serves to preserve the normal tension of the muscle-tendon. In his words, "we can best entwine the tendons by carrying the power-giving tendon through a slit of the power-receiving tendon, which has been left intact, and by making this position secure with a number of sutures." Vulpius was of the opinion that by the perfection of tenoplasty, the therapy

the transplant depends on accuracy of coaptation of tendon to tendon and on the delicacy with which the transfer is made. The surface cells of tendon differ structurally and functionally from cells of the deep strata. These superficial cells, frequently resembling cartilage cells in structure, seem intimately concerned with the gliding function, since their removal always causes adhesions. Mayer stated: "The surgery of tendons is most decidedly in the process of growth and much still remains to be said about the best method of free transplantation."

Gallie (39) (1921) observed many tendon transplantations that appeared to be successful for a time but ultimately proved to be failures because of separation of the tendon from its new point of insertion with bone. In his observations at a second operation in some of these patients he noted that a groove in which the tendon lay was lined with areolar tissue closely resembling the ordinary areolar sheath of normal tendon. Any adhesions existing between tendon and bone were long and slender, and offered no resistance to recurrence of the deformity, so he directed his attention toward the complete removal of all areolar tissue from the tendon at the point of attachment. In a modified technique the tendon ends were thoroughly scarified and split longitudinally before being laid in the groove in such a manner that the cut surface of the tendon was placed in contact with the bone substance instead of its superficial surface. More recently, some of the chips of bone removed were forced into the bony groove among the strands of the split tendon before the periosteum was closed over them.

Neuhof (40) (1923) held that although the experimental evidence is most encouraging, enough clinical material has not yet been accumulated to establish the superiority of living material over silk for the replacement of tendon defects. He believed that at "the present time" homotransplantation of tendon is not practicable and that tendon autoplasty has a limited sphere of usefulness. He seemed to have doubted an indefinite viability of tendon transplants, which Rehn stated as demonstrated, but was inclined to believe that the supposed retention of gliding surfaces may be new tissue laid down by the host and that the tendon graft itself ultimately is new replacement tendon from the host tissues, or at any rate the original tendon cells are replaced.

In Hauck's opinion (1924) tendons heal by the formation of scar tissue due to proliferation of the peritendinous connective tissue. A good blood supply was considered as being necessary. New tendon arises in the main from the sheaths surrounding the tendon stumps and the tendon itself plays no significant rôle (41).

Using silk thread in tendon transplantation, Lange was unable to prevent recurrence of the deformity by placing the limb in plaster of Paris for several weeks after operation. In 1927 he introduced the use of parchment as he found it more suitable in tendon transplantation. He believed that the parchment stimulates the development of a capsule. With the use of parchment he obtained excellent results (42).

In a report on tendon surgery before the French Congress of Surgery in 1929, Bloch and Bonnett (43) drew the conclusion that the tendon callus is in the main replacement tendon and that newly-formed tissue can always be distinguished from true tendon. It is possible that tendon cells may have only a minor role in the callus formation.

Koch (44) (1933) treated 101 patients with complicated contracture of the hands by tenolysis or tendon grafts, or a combination of the two methods during a period of 17 years. Grafts were used in 49 patients. He emphasized the necessity of securing a firm and permanent attachment of a tendon graft to the distal phalanx if the graft is to draw the finger into flexion. Mobility of the interphalangeal joints is essential to free

marked: "There is a splendid field for the application of tendon transplantation in cases of paralysis following anterior poliomyelitis."

As viewed by Lexer (32) in 1914, both fresh autogenous and homogenous tendon grafts heal in but they show an important difference in behavior at complete rest or under early functional demand. The tendon at rest is penetrated by scar, to which it is firmly attached. The functional stimulus in early motion incites strong proliferation of the peritenon, which fastens the tendon stumps to the place of suture; regeneration of tendon fibers occurs. Lexer considered that under functional demand it is possible for tendon grafts to substitute in transmitting muscle function, in supporting tendon dislocation, and in filling in tendon defects. In general, he held that fresh autoplastic tendon is the natural and best substitute. The homogenous foreign-body stimulus incites stronger encapsulation, due to which it may be better adapted as a substitute band with firmer adhesion than as a free-moving tendon.

In 1916 Biesalski and Mayer (33) described tendon transplantations on the foot, knee, hand, and elbows in humans. They demonstrated that an extensor hallucis muscle which had been transplanted for a paralyzed tibialis anticus muscle 3 years previously was almost exactly like a sound tendon in appearance and function. If good technique at operation was observed in transplanting a tendon with its gliding apparatus, its normal appearance and free gliding were maintained.

As reviewed in Chapter 24, Mayer (34) considered an attempt to reconstruct the normal gliding mechanism as the primary consideration. He applied the term "paratenon"[1] to the covering of loose areolar tissue peculiarly rich in elastic fibers. It is this tissue, lying between the tendon and the fascia, which permits free gliding motion of the tendon.

Sterling Bunnell (35) of San Francisco, a surgeon with great initiative and ingenuity, stressed atraumatic technique and discussed the causes of failure in repairing tendons in the fingers. Such failure was caused by traumatizing technique, median incision, obliteration of the pulleys, using methods which replace the gliding mechanism by adhesions, too much or too little postoperative movement, and crude suturing of the tendons. "The length of the tendon should be so arranged that when the origin and insertion of the muscles are approximated, as nearly as physiologically possible, the tension of the tendon will be zero." Bunnell preferred the use of thread spliced into tendon. He devised a clamp to hold the tendon fibrils firmly together, and a tendon stripper for freeing tendons from the tunnel, which will follow intimately along a tendon and plane the adhesions away from its surface.

Mayer regarded Bunnell's technique as superior in its simplicity and ingenuity to that used in any clinic here or abroad.

Steindler (36) (1918) emphasized the reconstruction of the gliding mechanism and the preservation of the mesotenoneum for the nutritional basis of the tendon. He revised the technique of reconstruction of the tendon sheath, basing it on the work of Biesalski and Mayer. In 1919 he reported on the results from applying his method of transplanting tendons which retain their attachment to their muscles, in 30 cases of paralytic deformities, postoperative observations being made from 2 to 16 months. The good results, he believed, were due to regard for the above mentioned factors. He also extended the period of fixation in a cast or plastic splint to one year or more (37).

Leo Mayer (38) (1921) believed that, in free transference of tendons, the vitality of

[1] Various writers use different terms for the slippery gliding mechanism covering the external surface of a tendon. Bunnell as well as Mayer used paratenon, but others used epitenon or peritenon.

the hip joint, distal transplantation of the trochanter to increase abductor efficiency; and reattachment of ruptures of the supraspinatus tendon at the shoulder, and also tears of the long head of the biceps in this area.

In Abbott's experience (1944) the results obtained by tendon transplantation in paralysis of the radial nerve have been very gratifying. In some cases patients have been able to extend the fingers with considerable strength, while in others the power of dorsal flexion was weak and the grasp ineffective despite careful and long-continued post-operative treatment. Additionally, arthrodesis of the wrist has been used to give early and more complete restoration of function in the latter cases. In complete lesion of the radial nerve the muscles best utilized are the pronator radii teres, the flexor carpi radialis, and the flexor carpi ulnaris. The insertion of the pronator radii teres is removed with the periosteum and passed through the tendinous portion of these muscles. The tendon of the flexor carpi radialis as well as the flexor carpi ulnaris is divided at the wrist joint and freed on the anterior aspect of the lower forearm. These muscles are then brought to the dorsal aspect of the wrist around the borders of the radius and ulna. Both tendons must be liberated for free transfer by division of the deep fascia of the forearm (49).

During a period of three years, Koch (50) (1944) operated on 46 patients with flexor tendon injury with division of the tendons within the digital sheath. In 14 of 41 cases of secondary repair it was possible to reunite the divided tendons without excessive tension. Primary union occurred in all but three patients. In repair of the index, middle or ring fingers the normal portion of the sublimis tendon was used as a graft. If the thumb or little finger was involved, or if the two tendons of any of the other digits were fused into a single tendon, it was necessary to secure a graft from the foot. The graft was carefully drawn through the sheath overlying the proximal phalanx, and was joined by end-to-end silk suture to the distal segment of the profundus. The tendon healed by formation of callus and with moderate, if temporary, thickening at the line of union. The space must be sufficient to allow the thicker callous portion of tendon to move back and forth without any constricting obstruction.

Young and Lowe (51) (1947) made a study of long term results in 28 patients in whom the tendons used and the methods of transfer varied greatly for destruction of the radial nerve or loss of the dorsal interosseous nerve high in the forearm. The results were excellent in 10 cases, good in 13, fair in 5. The degree of motion was sufficient to provide excellent function. Young and Lowe are inclined to feel that it is better to have wrist extensors somewhat stronger than flexors since slight extension of the wrist is necessary to produce a strong grasp. They recommended leaving the flexor carpis radialis intact provided the palmaris longus or some other active muscle is available for extension of the thumb. Numerous short cutaneous incisions are preferred to extensive ones. The flexor carpi ulnaris must be attached to the finger extensors sufficiently far from the dorsal carpal ligament to prevent impingement and fixation in flexing the fingers.

In 21 operations with free grafts and steel wire fixation, as reported by Kinmonth (52) (1947), the palmaris longus was used for all grafts except one, when the tendon of the flexor carpi radialis was used. Union with mobility is essential. The cases described and the series reviewed, according to Kinmonth, show that successful flexor tendon suture is possible in a reasonable proportion of patients even where the injury lies within the bounds of the digital sheath.

Littler (53) (1947) regarded small caliber

movement of the fingers. In the use of tendon grafts Koch's technical considerations are the preparation of a suitable bed for the graft, the technique of uniting it with the tendon, the method of attaching it to the distal phalanx if the graft is to replace the tendons of a finger or thumb, the construction of a gliding mechanism about the graft, the formation of new annular ligaments to hold it in place, the tension at which it should be sutured, and the degree of relaxation which should be maintained after operation. All these factors are important in securing satisfactory results.

During the last 10 years Peabody (45) (1938) had opportunity to perform 300 operations for tendon transposition in paralytic residuals. Of 215 patients checked at periods of 3 to 10 years after operation, a strongly-working transplant was observed in 90 per cent. Tendon transposition has proved to be the most useful procedure in restoring lost function, and frequently an imperative procedure in the prevention and control of imbalance deformity. Its utilization diminishes the frequency and extent of the stiffening procedures, sometimes as an adjunct and sometimes as a preferable substitute. Meticulous evaluation of the static, dynamic and kinetic conditions are as necessary for success as the operative technique.

Morganti (46) (1939) preferred the implantation of free autogenous tendon grafts above all other methods. He considered the long extensors of the feet the most practical and serviceable grafts. If possible, the tendon graft with its intact sheath was used by Morganti.

Wheeldon (47) (1939) reported on the use of cellophane to line joints in arthroplasties of the hips, knees, shoulders, elbows, wrists, and fingers. No irritation to the tissues was caused by the cellophane. In one patient with deformity from severance and wide separation of the tendon of the extensor pollicis longus, cellophane was used as a permanent tendon sheath. After the ends of the tendon had been freshened, they were united with braided silk. The sheath of cellophane was drawn beneath the distal end of the proximal fragment and under the proximal end of the distal fragment, the cellophane being wrapped around two fragments of tendon. There was excellent ability to extend and flex the thumb almost normally. The wound healed by first intention. There were no scar tissue and no adhesions.

Sutherland and Rowe (48) (1944) stated that tendon transplantation, after its initial enthusiastic use, in recent years has been used less frequently in favor of more extensive arthrodesing operation in many instances. They mentioned as some reasons for failure of tendons to work satisfactorily in their new position: fibrosis and fixation in the sheath during the long period of immobilization necessary for bone attachment to become firm; stretching of the healing area when the graft is sutured to soft tissue with resultant loss of efficiency and muscle tone; and stretching at the point of bone insertion of a tendon weakened by long immobilization. Sutherland and Rowe believe that early institution of function without external cast immobilization can be obtained by the use of metal nail fixation of the transplant to firm bone. In careful procedure with little trauma, much of the tendon sheath transplantation and special plastic pulley procedures may be neglected, since early institution of motion prevents formation of adhesions. They have used nail fixation successfully in lateral transposition of the anterior tibial tendon in talipes equinovarus and in replacement of the tibial tubercle with attached patellar tendon after operation on the knee joint. Other conditions mentioned are reattachment of the medial epicondyle of the humerus and the medial malleolus of the tibia; reattachment of the trochanter of the femur after approach to

tendon. Autogenous human tendon grafts in contact with tendon at one end had normal-appearing tendon cells and collagenous fibers when removed at intervals of 2 months and 4 months after transplantation. The end of the graft in apposition to the host tendon had become firmly attached. On the basis of these sections it was not possible to determine what specific cells took part in the healing process between graft and host tendon.

Graham (60) (1952) recommended that the transfer of tendons in the forearm or hands be done early so that the body and mind have not substituted new muscles to replace the injured ones. This refers to poliomyelitis as well as to muscle and nerve injuries that cannot be repaired. "It is necessary that all joints between the brain and the tip of the finger be stabilized for function to take place in the fingers." In transplantation it is desirable to use muscles that have an excursion similar to that of the muscles being restored. Muscles with adequate strength should be transferred so that the transplant will be "adequate to do the job which is assigned to it." The direction of pull of the transplanted muscle should be as near that of the tendon involved as possible. Graham added that it is not desirable to give a tendon two functions. The primary purpose of tendon transfers was to establish as near normal muscle control to the thumb and index finger as possible.

In very recent (61) work I buried separate segments of autogenous human tendon in abdominal fat and removed the grafts at intervals of 1, 2, 3, 4, 5, 6, 10, 14, 18 and 25 days. Tendon grafts buried for 7 and 8 months in abdominal fat were also removed and examined microscopically. All grafts were transplanted with paratenon. The grafts were segments of the palmaris longus tendon and were transplanted in burned patients who gave their consent for the experimental procedure.

The tendon grafts buried for one and two days were somewhat edematous, but the tendon cells appeared to be viable in stained sections. The usual host tissue reaction was present around the periphery of the grafts, consisting of new blood vessel formation, dilatation of established blood vessels, and a moderate number of lymphocytes and polymorphonuclears. Young fibroblasts were active everywhere in the host tissue. The blood vessels in the tendon grafts had normal-appearing endothelial cells and *the lumina of the blood vessels were empty and collapsed* in most instances. In some the capillaries contained a few red cells, which apparently had been trapped within the vessels. The stroma of the graft contained occasional polymorphonuclears and lymphocytes. These probably had also been trapped in the blood vessels and had infiltrated the graft by diapedesis through the blood vessel walls.

Blood vessels in some tendon grafts buried for 3 days contained normal-appearing red blood cells in rather large numbers, which indicated that anastomoses between host and surviving graft blood vessels had occurred and that active circulation had been established. Numerous engorged blood vessels were present in tendon grafts buried for 4 and 5 days and these contained many normal red blood cells, polymorphonuclears, and lymphocytes. *In many areas diapedesis of white blood cells from the graft vessels into the tendon graft structures had occurred.* These dense collections of cells were identified as polymorphonuclears, lymphocytes, plasma cells, and eosinophiles. In some sections there was a general absence of host-cell diapedesis. All tendon cells and fibroblast cells in the graft appeared viable although the collagenous fibrous structure was edematous. It was not possible to differentiate tendon cells from stromal fibroblast cells. In general, the cell population appeared more numerous than in normal control tendon but mitosis of cells

tendon grafts as preferable in secondary repair of flexor tendon because of loss of interphalangeal joint flexion. Restoration of a free, strong, gliding tendon gave excellent results in 50 per cent of 36 free flexor tendon grafts placed according to the principles of Bunnell.

Van Demark (54) (1948) noted that tendon transplants undergo an early central avascular necrosis, then become vascularized more rapidly and after a period of four to five weeks they have considerable strength. During the period of vascularization the graft is swollen, later it contracts and resembles a normal tendon both grossly and microscopically. His procedure in injury of digital flexors is to attach a tendon graft to the base of the terminal phalanx of the finger, pass it under the intact or reconstructed annular ligaments to the palm, where it is sutured to the tendon of either the profundus or sublimis, usually the former tendon. In a case of deep penetrating wound over the metacarpal head with division of both flexor tendons, Van Demark replaced the flexor tendon with the long extensor tendon of the left fourth toe. Flexion and extension of the right index finger were present nine months postoperatively.

Kirklin and Thomas (55) (1948) reported that if the palmaris longus is thin or weak and if the short extensor of the thumb is attenuated or exerts a poor action on the thumb, they remove the flexor digitorum sublimis tendon and insert it directly into the bone of the thumb.

Flynn (56) (1949) believes that tendon grafting can restore function in a hand that has a loss of flexor action provided there are good circulation, normal joint function, and normal sensation in the affected finger. A tendon transplanted with the paratenon glides better than one grafted without it. In the cases reported by Flynn tendon grafting restored the pinch and grasp mechanism in hands with loss of flexor function after acute suppurative tenosynovitis and trauma.

In a patient with spastic paralysis of the forearm, as reportedly by Nicod (57), of the *Hospice orthopédique de la Suisse romande* (1949), tenotomy of the contracted muscles was performed because of recurrent contractures, and the anterior cubital was transplanted on the extensor tendons of the fingers, and the round pronator, on the radialis. In another case of an infant with spastic paralysis, the tendons were elongated. Nicod states that the "paralyzed muscles were reanimated" and the forces equalized by the grafts. He thinks that in these two patients a functional amelioration and appreciable esthetic effect were obtained.

In a series of flexor tendon grafts in fingers and thumb, inserted because of damage to tendons, Boyes (58) (1950) found the influencing factors to be the cicatrix from injury or infection, stiffened joint, trophic changes, and damage to more than one digit. He excised the scar and thickened sheath tissue, and used the Bunnell type of stainless steel sutures. In his experience the use of the palmaris longus tendon as a graft has given better results than the use of other tendons. In the thumb a graft from the musculotendinous origin is preferred. In 25 per cent of digital flexor tendon grafts flexion is complete, the pulp of the finger reaching the distal crease of the palm.

Peer and Walker (59) in 1951 reported the microscopic findings in autogenous human tendon grafts buried in contact with unlike tissue (abdominal fat) and with like tissue (tendon). The tendon cells in a graft buried for 14 days in fat showed little, if any, degenerative change, and the collagenous fibers were quite normal in appearance. The tendon cells and the arrangement of the collagenous fibers were entirely normal in a graft buried for 7 months in abdominal fat and grossly the graft appeared like normal

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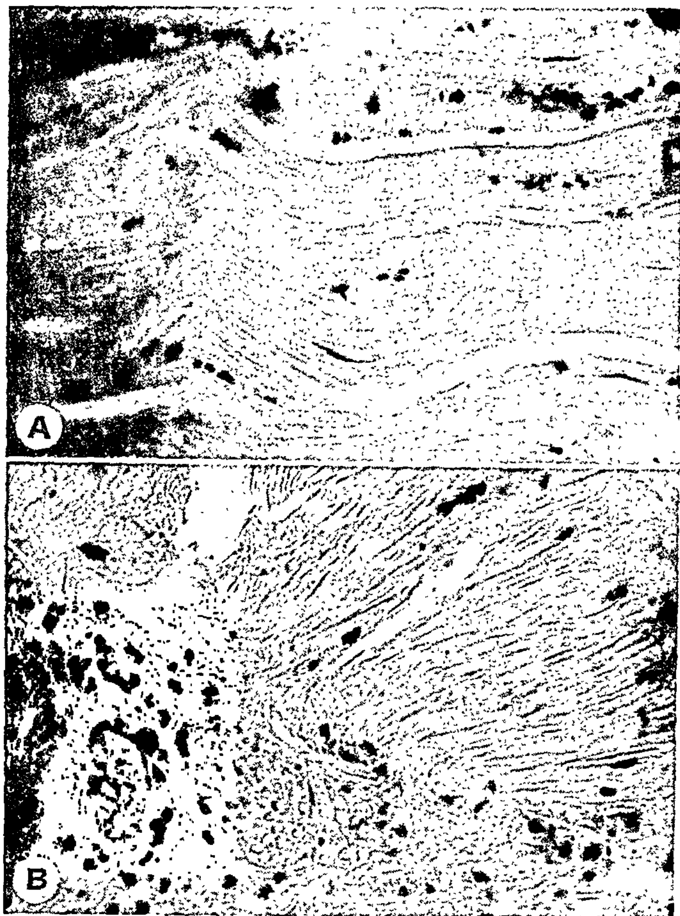


FIG. 107. Autogenous human tendon grafts buried for 3 days in abdominal fat.

A. In this tendon graft the blood vessels in the graft were collapsed and empty, indicating that anastomosis between host and graft blood vessel had not as yet taken place. Some edema is present as shown by the separation of the groups of collagenous fibers. The tendon cells appear more numerous than those seen in control sections of tendon but none of the tendon cells show evidence of degeneration or death. Note the complete absence of fibroblast invasion of the graft from the surrounding host tissue. ×340.

B. A different tendon graft buried for 3 days. In this transplant anastomosis between host and graft blood vessels has occurred, and two blood vessels distended with red blood cells and a few white blood cells are clearly seen. White blood cells have passed through the blood vessel walls and formed small collections in the graft structure. These will return to the blood vessels again, as shown in tendon sections buried for 14 days. Tendon cells and matrix are normal and there is no evidence of invasion of the graft structure by host fibroblasts. ×340.

was not actually seen. *There was no evidence of mass invasion of the graft structure by host fibroblasts.* Grafts buried for 4, 5 and 6 days had about the same histological picture.

The graft buried for 10 days showed considerably less edema and only a few areas occupied by extravasated white blood cells. The collagenous fibers were more compact and the tendon cells were normal in appearance, as were the fibroblasts in the stroma of the tendon. Apparently the graft had "settled down" and was becoming adjusted to its new host site.

It should be emphasized that there were no evident areas of invasion of the tendon grafts in this series by fibroblast cells from the host tissue. Since fibroblasts do not travel through the vascular system, *it is therefore probable that the tendon cells in the graft survived as such and were not replaced by host tissue fibroblasts* up to 10 days.

The graft buried for 14 days showed little host-tissue reaction and that buried for 25 days showed practically none. There was a complete absence of invasion of the graft structure by host-tissue fibroblasts and the tendon cells in the graft had therefore probably *survived as such* unless they were replaced by the fibroblasts in the connective-tissue stroma of the graft. These fibroblasts also probably survived as living cells and could not readily be differentiated from the tendon cells. There was an absence of extravasated white blood cells in the graft

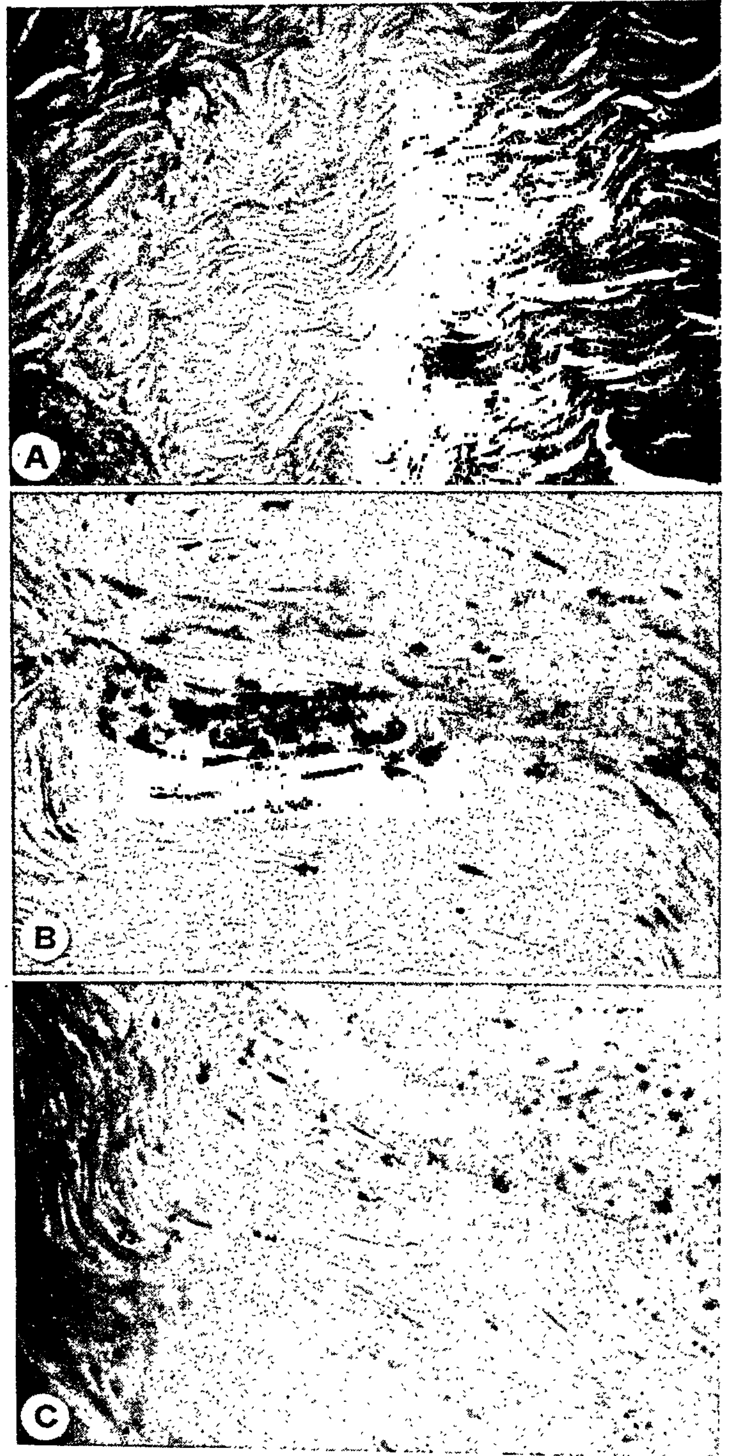


FIG. 108. Autogenous human tendon grafts buried for 4 days in fatty tissue.

A. Note blood vessel engorged with red blood cells, normal appearance of collagenous fibers, and scattered fibroblast cells. There is a complete absence of any obvious infiltration by host fibroblasts. The endothelial cells lining the blood vessels survived in this 4-day graft and circulation was established through end-to-end anastomosis between host and surviving graft blood vessels. The stroma of the graft is somewhat edematous. ×100.

B. Higher power magnification of another area of the same 4-day tendon graft. Note blood vessel engorged with blood cells and normal appearance of tendon cells and collagenous fibers. No dead or dying tendon cells were seen. ×430.

C. High power magnification of a different autogenous tendon graft also buried for 4 days in fatty tissue. In this graft anastomosis has occurred between host and graft blood vessels and the large blood vessel shown in the photograph is engorged with red and white blood cells. Some of the white blood cells have infiltrated the graft through the walls of the blood vessel. Tendon cells and collagenous fibers are normal. ×430.

structure but blood vessels were numerous as compared to those in control tendon.

The tendon grafts buried for 7 and 8 months appeared exactly like normal tendon, but there seemed to be an increase in total cell population when compared to that in control tendon.

SUMMARY COMMENT ON AUTOGENOUS AND HOMOGENOUS TENDON GRAFTS

In this review the following anatomical terms will be used. 1) *Paratenon* is the loose areolar tissue covering the tendon which provides a gliding mechanism for the tendon.

2) Where tendon sheaths are present, *epitenon* is the term denoting the areolar tissue in contact with the tendon. 3) *Endotenon* is the connective-tissue stroma between the various bundles in an individual tendon. The fibroblast cell in this stroma or endotenon is a living entity (separate from the tendon cells), which may participate in the wound healing of severed tendons and in the healing of free grafts in contact with host tendon.

The earliest interest in tendons arose through the necessity of reuniting severed tendon ends. The second concern was the possibility of transplanting a tendon attached to its active muscle in some new location and, later, surgeons became interested in utilizing a free tendon graft to bridge a defect in tendon.

All these procedures are important clinically and all depend on the preservation of a gliding mechanism or the substitution of such a mechanism to permit free movement of the tendon.

Histological studies at the site of healing in severed tendons have been made on animals by numerous investigators, and similar animal experiments have been carried out at the site of healing in free tendon grafts in contact with host tendon and in contact with unlike tissues (muscle and fat). *The results of this experimental work on animals, as described in Chapter 24, are controversial.* Since nearly all of our knowledge regarding the healing of severed human tendons and free tendon grafts is based on animal experimental work, the opinions concerning tendon healing in the human reflect this confusion.

Regarding the survival of the tendon cells and their matrix in autogenous grafts there is also conflicting opinion, *which is likewise based on animal experimental work.*

Healing of Severed Tendons

There are apparently two schools of thought in the literature regarding the healing of severed tendon, based mostly on the results of animal experiments.

One group of investigators believe that the scar or fibrous tissue union between the severed tendon ends arises through the activity of infiltrating fibroblast cells from the surrounding host connective tissue. The investigators who subscribe to this viewpoint do not believe that the tendon cells in the proximal or distal severed tendon ends or fibroblast cells in the paratenon or tendon sheath take part in the healing process. Apparently the fibroblast cells in the stroma around tendon bundles in an individual tendon are ignored as possible agencies in the reparative process.

Another school of thought emphasizes that early union occurs from a proliferation of cells in the paratenon or tendon sheath, which is later supplemented by active growth of the tendon cells in the severed tendon ends; these "bridge the gap" by new formation of collagenous fibers. Again, no mention is made of the fibroblast cells in the connective-tissue stroma of the severed tendon. One cannot definitely state which of these viewpoints is true.

Autogenous Grafts

In free tendon grafts there are other factors which must be considered in addition to healing of the host tendon ends with the free ends of the tendon graft. *Do the various cellular elements in free grafts survive as living entities, or are they replaced by ingrowing cells from the host tissue?* One notes in the literature that investigators speak only of the survival or replacement of the tendon cells. One should also consider the fate of the fibroblast cells in the stroma and that of the blood vessels in the graft, with their lining endothelial cells. Additionally, it would be interesting to know the fate of the collagenous and elastic fibers in tendon grafts.

The opinions expressed in the literature regarding the behavior of free tendon grafts

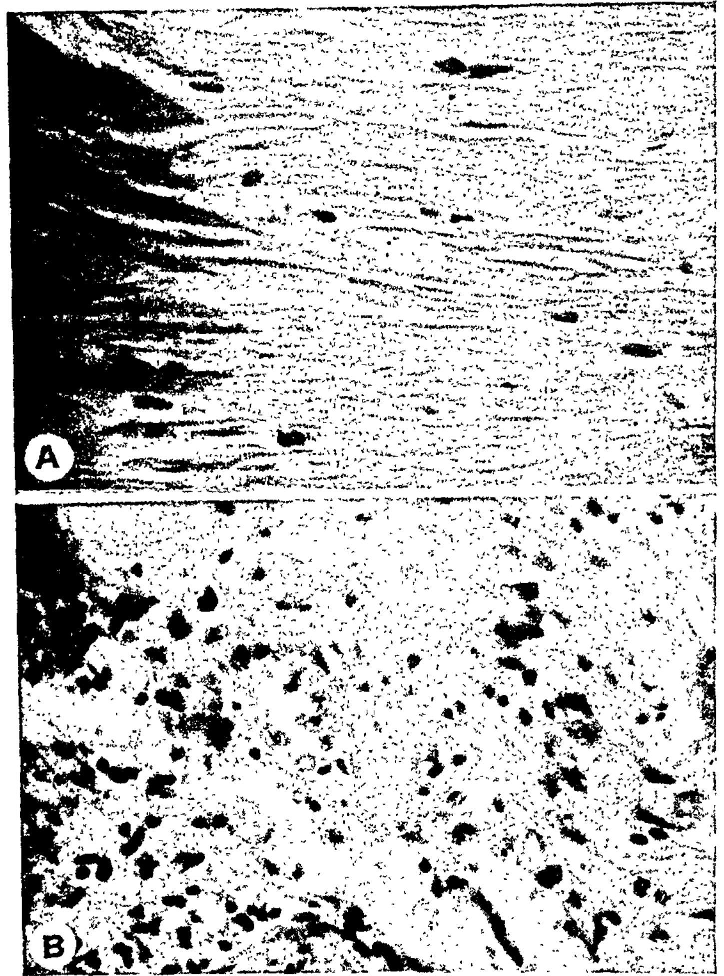


FIG. 109. Autogenous human tendon grafts buried for 10 days in abdominal fat.

A. In this graft the tendon cells with large dark staining nuclei are seen to be normal in appearance, with a complete absence of any degenerative changes. These are probably viable cells and there is no evidence in this 10-day section or in earlier sections that host fibroblasts have replaced the original tendon cells. In this graft there is a complete absence of white blood cell infiltration although this may have been present in the graft earlier. The collagenous fibers are normal, the edema has subsided and blood vessels in the graft are small.

B. A different tendon graft buried 10 days shows a more prolonged dilatation of blood vessels and a persistence of cellular exudate in the substance of the graft. Autogenous tendon grafts buried for 20 days or more all show an absence of this cellular infiltration. The tendon cells in this graft are normal and there is an absence of host fibroblast invasion.

in humans are largely based on histological examinations of tendon grafts in animals, as previously noted. Some believe that infiltrating host cells replace the tendon cells in free tendon transplants. These investigators do not make any commitments regarding the survival of the fibroblasts in the stroma, the endothelial cells lining blood vessels in the graft, or the fate of the collagenous and elastic fibers. Others hold that the tendon cells in free grafts survive as living cells, which not only retain their collagenous fiber matrix but also participate actively in the healing process between graft and host tendon.

In a consideration of the behavior of free autogenous tendon grafts we are confronted with two problems: the nature of the healing process; and the fate of the cells and intercellular material in the graft.

In the author's opinion it is not possible to state the exact nature of the healing process between the host and graft tendon at this time. Obviously the fibroblasts and their accompanying blood vessels grow into the space between the graft and host tendon. One sees them in fixed and stained sections examined at various intervals of time, the whole cycle of healing resembling that which occurs between host and graft dermis in free skin grafts. Eventually the free tendon graft becomes joined to its host tendon ends by scar formation, and the paratenon or tendon sheath of the graft joins the paratenon or sheath of the host tendon.

It is not known just where the fibroblasts which take part in tendon wound healing come from, and every possible source has been predicated by different investigators. The main theories are as follows: 1) Healing occurs from cells in the paratenon or sheath when these are present; when they are ab-

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The opinions expressed in the literature regarding the behavior of free tendon grafts

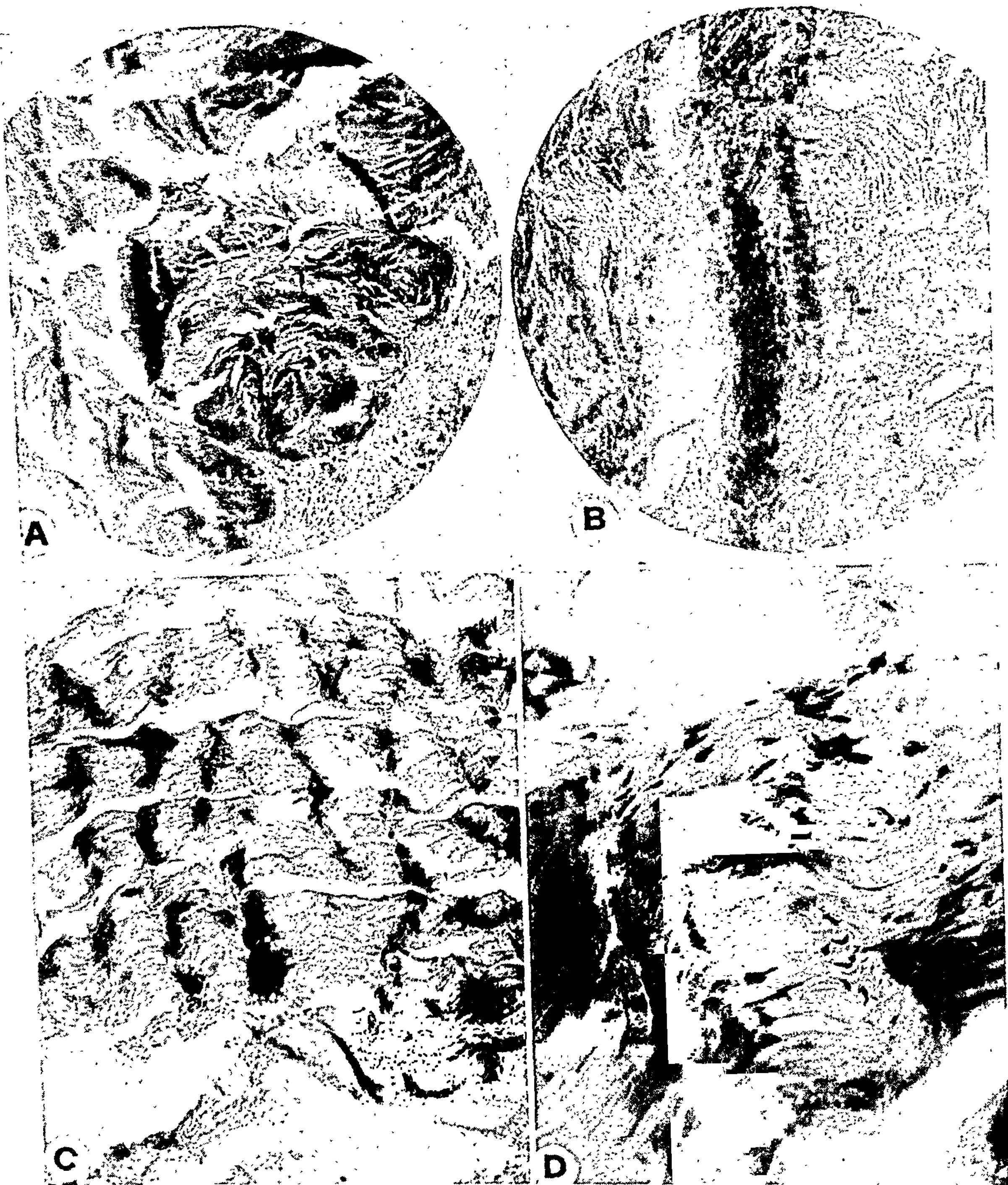


FIG. 110. A. Tendon graft (autogenous human) buried in fat 14 days. Structure is normal and tendon cells appear normal (cross section).

B. High power magnification of tendon graft buried in fat 14 days. Note living tendon cells with their collagenous fibers.

C. Tendon graft buried in fat for 7 months. The appearance is that of normal tendon (longitudinal section).

D. High power magnification of tendon graft buried in fat for 7 months. Note living fibroblasts separated by normal appearing bundles of collagenous fibers.

sent, healing occurs through the agency of ingrowing fibroblasts from the surrounding host connective tissue. 2) Healing always occurs from ingrowing fibroblasts from the host tissue, which grow into the space between the graft and the host tendon and join them together as well as the severed ends of the paratenon or tendon sheath. 3) The fibroblast cells in the paratenon or sheath form an early union between the graft and host tendon. Later the tendon cells themselves, both in the graft and in the host tendon, proliferate and participate in the process of healing. This latter conception predicates that the tendon cells in the graft survive as living entities.

One must admit the great difficulty, on the basis of fixed and stained sections, of determining just where the proliferating fibroblast seen in tendon wound healing originates. Perhaps future work with radioactive substances will enable investigators to tag all cellular elements in the graft and thus differentiate those marked graft cells from unmarked cells which would represent host entities.

Alternately, however, *one can make more definite statements regarding the behavior of cells and intercellular material in the tendon graft itself.*

The acceptable facts stated by others and the author's own observations indicate that the probable fate of autogenous human tendon grafts transplanted with their slippery paratenon covering is as follows: Anastomosis occurs between the severed ends of host blood vessels and the open ends of vessels in the graft, and blood circulation is established in the transplant in about 3 to 4 days. The blood vessels in the graft survive as such, with viable endothelial cells lining the intima of vessels. These surviving blood vessels in the graft enlarge and probably give rise to new blood-vessel sprouts, and in certain areas of the graft collections of polymorphonuclear leukocytes, plasma cells, eosinophiles, and lymphocytes are often seen. These gain access to the intercellular substance of the tendon graft by diapedesis through the walls of small blood vessels and may often be noted in the process of passing through these vessels. A similar cellular exudate is present in the host tissue adjacent to its contact with paratenon. There is a proliferation of fibroblasts in the host tissue but it appears to invade only small portions of the outer surface of the paratenon in areas where the paratenon or tendon has been injured by operative trauma. There is *no evidence of mass invasion of the graft structure by host fibroblasts*, and one does not expect fibroblasts to travel through the blood vessels like lymphocytes, polymorphonuclears, and the like. Thus it is probable that the tendon cells and stromal cells in tendon grafts survive as such, since there is no demonstrable agency which replaces them. One can of course argue that the stromal fibroblasts survive and replace the tendon cells but there is no direct evidence that this occurs.

There is a proliferation of tendon cells or stromal fibroblasts within the tendon graft. It is not possible, in the author's experience, to clearly differentiate tendon cells from the fibroblast cells in connective-tissue stroma of the grafts. Tendon grafts buried up to 18 days may still be edematous, but in 25 days the edema has largely subsided and the collagenous fibers appear quite compact. In 30 days the graft appears much like normal tendon excepting for a larger number of blood vessels in the graft structure and a larger number of cells. Grafts buried in fat and removed after seven and eight months appear grossly and microscopically as normal control tendon, with about the normal sparse blood vessel supply. Penetrating capillary growth into the paratenon was not observed in the author's series of tendon grafts, *which does not mean that it did not occur to some extent,* as in surface skin grafts,



FIG. 112. Homogenous human tendon graft fixed in alcohol and buried 1 month in abdominal fat. Note intense cellular infiltration of the graft structure and the presence of numerous giant cells. Some of the tendon cells appeared rather normal in fixed and stained section. One cannot say whether these were the original tendon cells well preserved by the alcohol or new host cells which had infiltrated the tissue. The graft, grossly, appeared like tendon. ×80.

scopic findings in homogenous human tendon grafts, it does not seem presumptuous to present an unpublished report on the subject.

The author (62) buried three homogenous human tendon grafts, preserved in 70 per cent alcohol for two months or more, in human abdominal fat. The grafts were removed after 1 month, 2 and 6 months and examined in fixed and stained sections. All three grafts could be easily recognized as tendon on gross examination. Microscopic examination of the graft buried for one month demonstrated a large amount of cellular exudate in the host tissue surrounding the tendon graft and within the substance of the tendon. The cells consisted of polymorphonuclears, eosinophiles, cartwheel plasma cells, lymphocytes, and numerous giant cells. A large number of blood vessels were present in the graft, and the collagenous fibers were separated by edema and *large wedge-like ingrowths of fibroblasts from the host tissue* (a finding not observed in tendon autografts). Fibroblasts were present between the collagenous bundles but these were scattered and did not have the columnar arrangement of normal tendon cells. The grafts buried for 2 and 6 months showed about the same histological pictures as the graft buried for one month.

Based on the known facts at this time, the inference is that all of the cells in fresh homogenous tendon grafts probably die after transplantation. In both fresh and preserved homografts the collagenous fiber bundles and sparse elastic fibers appear to remain for some time (up to 6 months in the author's experience with dead tendon homografts). Blood vessels from the host tissue invade the graft and new fibroblast cells appear between the collagenous bundles. *A very large cellular exudate persists within the graft and outside it, indicating that the host tissues have not accepted the foreign homograft.* Extensive invasion by fibroblasts from the host tissue is noted. The homograft is recognizable grossly as tendon six months after transfer but has not been removed and examined grossly and histologically after long intervals of time.

HETEROGENOUS TENDON GRAFTS

The grafting of tendon from animal to man, surprisingly, was attempted successfully (according to reports) in the latter part of the nineteenth century and yet since that time only a few scattering descriptions of such trials have appeared in the literature. In 1882 Helferich (63), at the Policlinic in the University of Munich, removed a tumor from the upper half of a biceps brachii in a patient and transplanted the biceps femoris from a dog into the defect. The paramuscular connective tissue was left on the transplant and several large vessels were retained. The wound healed by primary intention.

and in fat grafts. The blood circulation appears to take place through established vascular channels in the graft after anastomoses have taken place between host and graft blood vessels about three days after transplantation. *Apparently most of the positive factual evidence favors the viewpoint that the tendon cells, stroma cells, and vascular endothelial cells in autogenous tendon grafts tend to survive transplantation as living cells, associated with their normal matrix structures.* No one knows whether the tendon cells retain their original collagenous fibers or manufacture new ones. At any rate the original elastic fibers in the tendon graft are probably retained, since these are not known to be replaced in any tissue.

That the tendon cells in free autogenous tendon grafts are gradually replaced by infiltrating host cells has not been verified by factual evidence. Certainly, the hairs and glands in skin grafts are not replaced by infiltrating host fibroblasts, nor is there positive evidence that the fat cells in a human fat graft are replaced by host cells which are alleged to take on fat and become fat cells. *Past and present investigators appear to have been unduly impressed by the theory of host tissue replacing free autogenous grafts in such a clever way that the counterfeit graft is an exact duplication of the original.* This probably does occur in dense autogenous bone grafts in contact with bone, but it has not been demonstrated in other commonly used free autogenous grafts such as cartilage, fat, skin, nasal bone, fascia, muscle, and *lastly tendon grafts.*

The factual evidence regarding free autogenous tendon grafts indicates that all cellular elements in the graft show a remarkable ability to survive as living cells. This tendency to survival of the cells in free autogenous human grafts is a dominant trend, the one definite exception being its absence in free muscle grafts. In these the muscle cells always die but the graft is replaced by fibrous tissue and not as muscle.

Homogenous Grafts

Sterling Bunnell, Sumner Koch and other authorities on tendon grafting use only autografts. Like similar type of bone grafts, homogenous tendon grafts, both living and preserved, must be replaced by the host tissue to be permanently serviceable. The available evidence indicates that homogenous tendon grafts give rise to a greater amount of reaction in the surrounding host tissue than occurs in autografts. There is evidence that some degree of replacement of the tendon by host tissue does take place but further experimental work is required to determine the nature and extent of this replacement, and the behavior of the grafts over long periods of time. In view of the scarcity of articles describing the micro-



FIG. 111. Boiled autogenous human tendon graft buried for 18 days in subcutaneous fat of leg. Note areas in graft where liquefaction has occurred. Under higher magnification it was noted that definite location within the graft was occupied by infiltrating host fibroblasts, host exudate cells, and ingrowing host blood vessels. ×80.

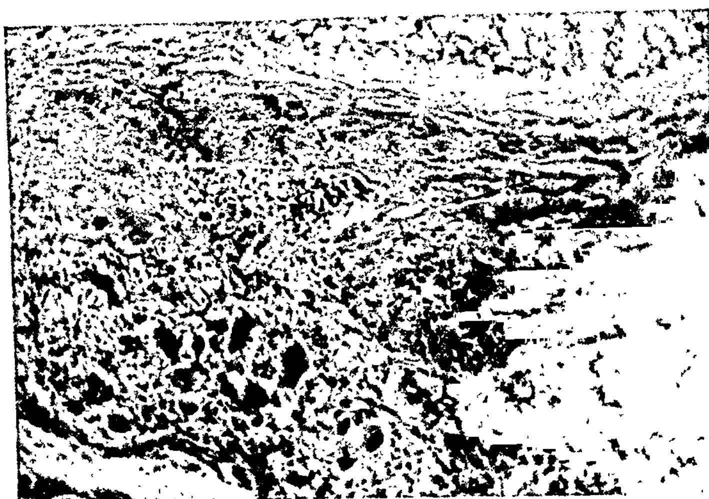


FIG. 112. Homogenous human tendon graft fixed in alcohol and buried 1 month in abdominal fat. Note intense cellular infiltration of the graft structure and the presence of numerous giant cells. Some of the tendon cells appeared rather normal in fixed and stained section. One cannot say whether these were the original tendon cells well preserved by the alcohol or new host cells which had infiltrated the tissue. The graft, grossly, appeared like tendon. ×80.

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Active motion of the limb was strong and without pain or other disturbance.

In 1886 Peyrot (64) succeeded in transplanting a tendon from a dog between the ends of a severed flexor tendon of the middle finger in a fourteen-year-old boy. The transplant healed in successfully. In 1887 Monod (65) reported transplantation of 5 cm. of rabbit tendon, which had been immersed erroneously in hot sublimate solution so that it had stiffened through albumin coagulation, into a patient. The united tendons became capable of function. In an experiment similar to Monad's, Hoffa (66) obtained healing in of a rabbit tendon in a human but not with a satisfactory functional result.

In an infection of the radial sheath of the hand in a woman, as reported by Romant (67) (1916), the thumb became fixed in a poor position. The thenar mass was freed and the proper flexor muscle was exposed. A tendon from a rabbit's paw was introduced under the fibromuscular layer of the thenar eminence. In 7 days there was perfect union, followed by healing in 20 days, with tendinous restoration. The thumb was straight; the cicatrix was supple. Function was reestablished permitting opposition, and movement of the thumb to the palmar surface of the hand.

In a case of injury reported by Auvray (68) (1919) the tendons showed short extension and the long abductor of the thumb was sectioned. Using Sencert's procedure, he placed a graft of dead animal tendon between the two ends of the sectioned tendon and fixed it at each end by silk thread. Auvray noted that the tendon sheath has been reconstituted around the graft. So far the result is very satisfactory, the movement of extension of the first and second phalanx being complete, but the functional restoration of the tendon of short extension shows that the movement of abduction is still slightly limited.

In five patients Jalifier (69) (1920) used fragments of tendon from the hind leg of the calf or tendons from the legs of a dog to rapair tendinous defects in the hands. The grafts were fixed in alcohol at 90°C. for 8 to 15 days, then preserved in alcohol at 60°C. The results obtained were unequal, integrity of movement being restored in some of the patients.

Hutchison (70) (1923) is said to have referred to kangaroo tendon used in correcting hernia. When the sutures were examined two years or more after operation, they could still be recognized. Microscopically they had become normal fibrous tissue, part of the living structure of the host.

In a patient with a wound followed by infection of the wrist and suppuration, reported by Nageotte (71) (1926), flexion of the fingers remained irreducible. Six grafts of tendon were removed from a dog and preserved in alcohol for one month, and then implanted, resulting in union by primary intention and without an inflammatory reaction. At the end of 8 months this patient was able to extend all fingers completely and flex all phalanges almost completely except the medius, which had slight limitation. Nageotte observed that during the entire period of rehabilitation and revascularization, dead tissue must be completely surrounded by healthy tissue. The tissue of the host could reconstitute, around the tendon grafts, a complicated system of serous membranes equivalent to that which existed before the lesion developed. Nageotte concluded that the absolute freedom of all movements could not have been restored if the gliding of the tendons had not been assured by the formation of suitable serous membranes.

In a case of injured medial meniscus with torn crural ligaments Micheli (72) (1933) made a canal in the femur extending from the medial side to the intercondyle space and a similar canal in the tibia. The joint was fixed by passing a kangaroo tendon

through the two canals. The knee was immobilized for 30 days and kept bandaged for another month. After three months postoperatively the wound healed, with good results.

Strickler (73) (1938) noted that chromicized beef tendons prepared in plates, cuffs, and pegs did not show on a roentgenogram, would remain in position for 60 to 90 days, had sufficient strength, and maintained reduction of the fracture if properly introduced. He advised use of chromicized beef tendon only if an open operation is indicated and when other, more simple methods have failed. In 10 patients the beef tendon served in splinting and supporting the lines of fracture perfectly and was absorbed at the end of 4 months. Strickler considered that in one patient the beef graft was in no way responsible for the non-union of the fracture, as previous and subsequent treatment by different methods were also failures.

SUMMARY COMMENT ON HETEROGENOUS TENDON GRAFTS

The literature on heterogenous as on homogenous tendon grafts in humans consists largely of clinical reports regarding the function of the grafts after transfer rather than microscopic examinations of the grafted tendons. This is quite understandable since it would not be practical to remove grafts which were functioning in patients as well as many of the investigators described.

Some of the reported results following the use of heterogenous and homogenous tendon in humans are so satisfactory that they strain the credulity of the surgeon who has often been disappointed by the degree of functional improvement following the use of autografts.

There are no reliable reports on the late histological findings in preserved heterogenous tendon grafts transplanted in humans. The ultimate fate of heterogenous as well as homogenous tendon grafts in humans cannot be stated at this time but a clearer picture will probably be available in the near future since much experimental work is under way. Thus it may be said that the transplantation of heterogenous tendon grafts in humans is still in the field of experimental surgery (in the year 1955).

Drawings Indicating Usual Behavior of Tendon Grafts in Man

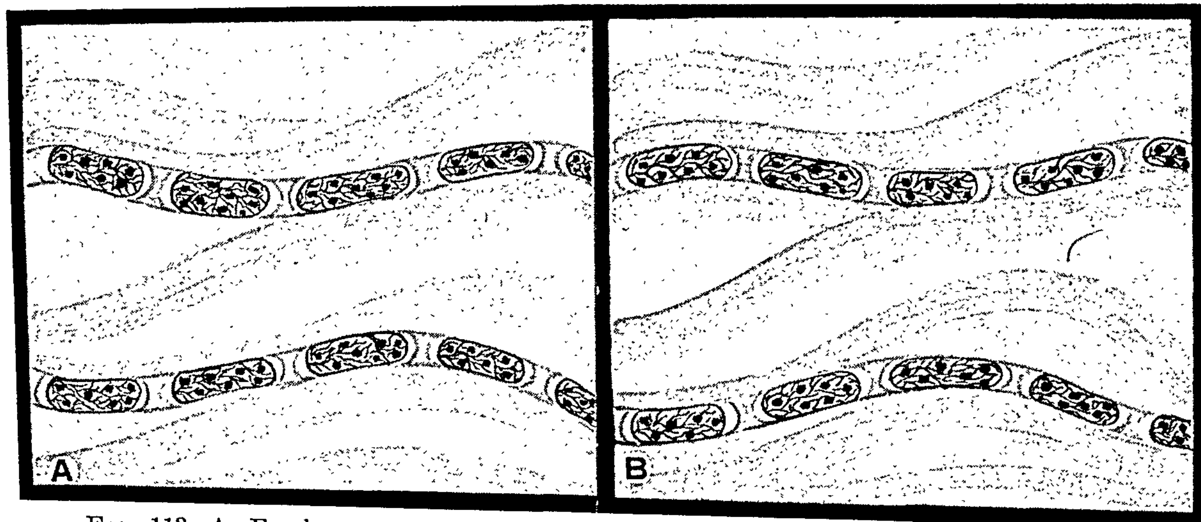


Fig. 113. A. Fresh autogenous human tendon grafts in contact with tendon. B. The tendon cells survive and retain their collagenous fiber matrix. Grossly and microscopically the transplant appears as normal tendon tissue.

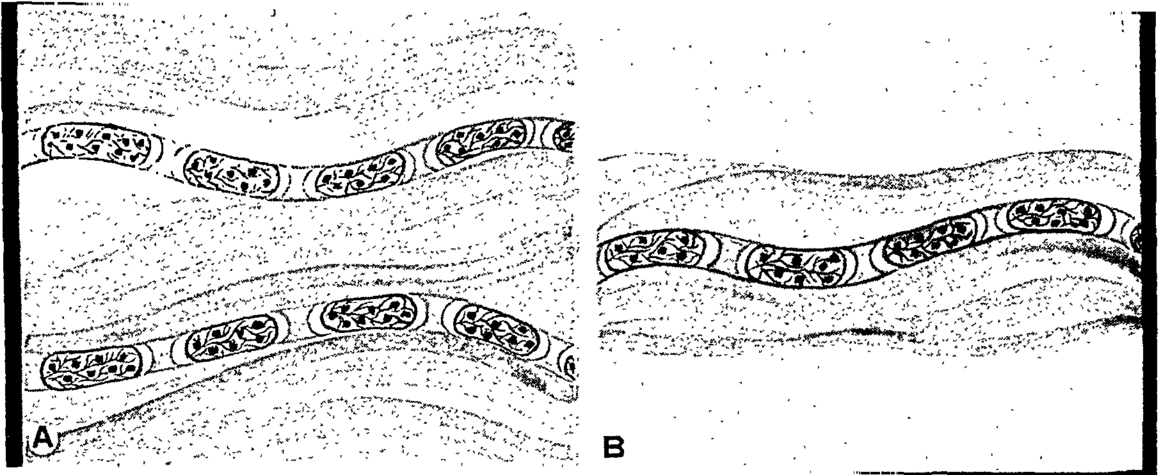


Fig. 114. A. Fresh autogenous human tendon grafts in contact with fat. B. The tendon cells survive and retain their collagenous fiber matrix. Many authorities state that the bulk of the tendon graft becomes reduced and that it assumes a yellowish color due to atrophy from disuse. In our series the tendon grafts appeared like normal tendon and did not seem to be reduced in size from gross estimation until 8 months following transplantation.

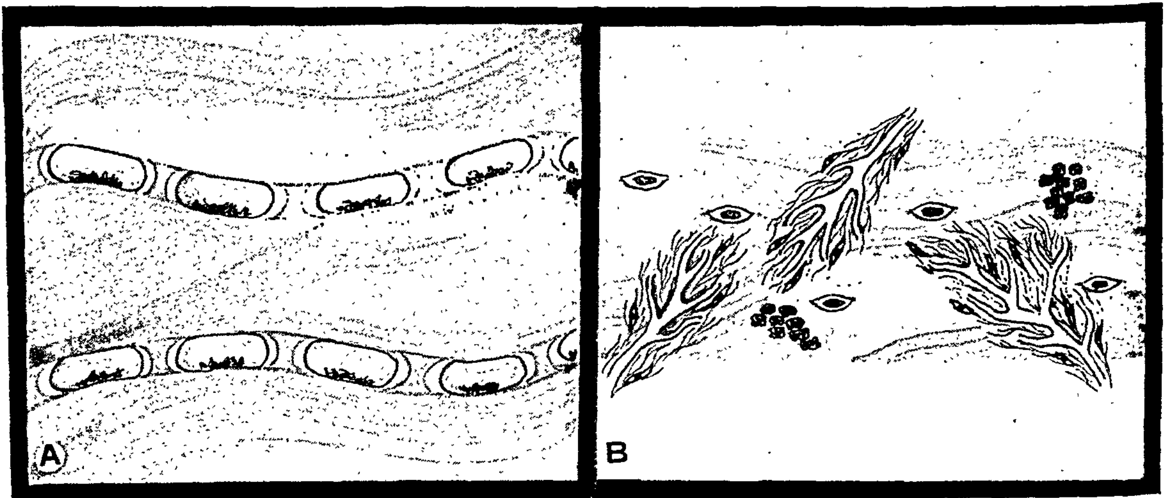


Fig. 115. A. Preserved or fresh homogenous human tendon grafts in contact with fat or tendon. B. The tendon cells in fresh grafts do not survive long as viable structures. Host blood vessels penetrate the graft accompanied by host fibroblasts. Large collections of exudate including giant cells are present in the graft with fibroblasts and histocytes scattered throughout. The collagenous fibers in both fresh and preserved homografts persist for considerable periods of time. The ultimate fate of these homografts is not definitely known.

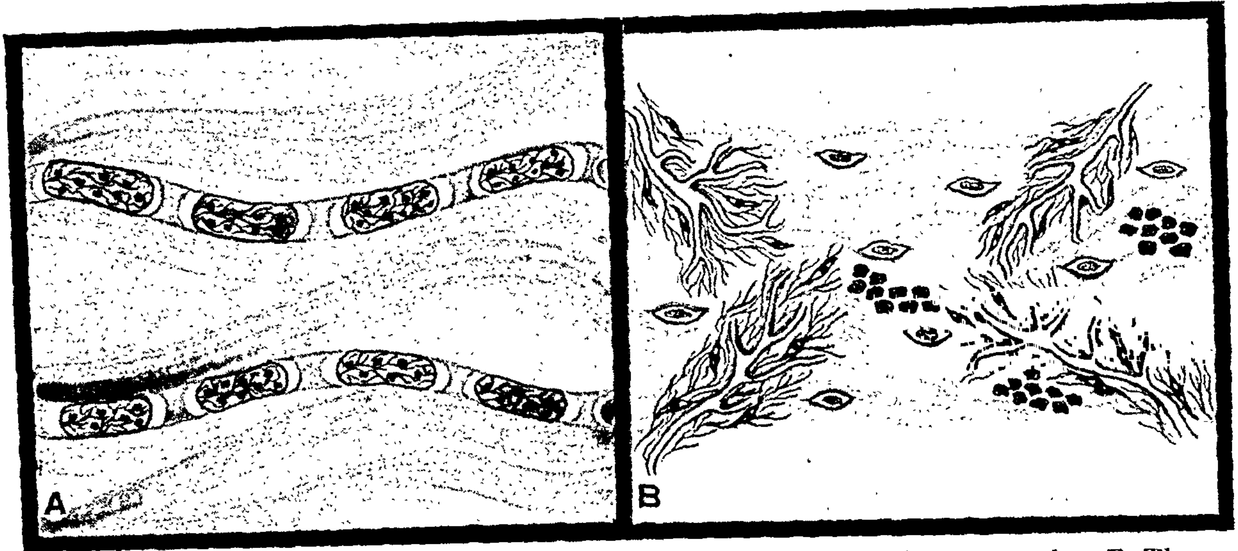


FIG. 116. A. Preserved heterogenous tendon grafts in contact with fat or tendon. B. The behavior of heterogenous grafts is similar to that of homogenous grafts excepting that the host reaction in rejecting the graft is more severe. The ultimate fate of heterogenous tendon grafts in humans is not known.

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CHAPTER 26

Clinical Use of Fascia and Tendon Graft

CLINICAL USE OF FASCIA GRAFTS

Undoubtedly, fascia grafts in the past were used in correcting conditions for which they were not suitable. For instance, fascia is a tissue which is not normally found on the body surface or on the exposed surfaces of body cavities such as the stomach, bladder, lumen of glands and so on. In these locations fascia, anatomically, is always protected by covering cells. Generally, therefore, fascia, like tendon, should be used only as a completely buried graft and not transplanted in locations where one surface of the graft is exposed. One is inclined to believe, however, that autogenous fascia grafts may again become popular for the repair of deficiencies in blood vessels instead of homogenous blood vessel segments and "tolerated foreign-body" pipes or wrappings. Fascia grafts used to repair blood vessels are not actually exposed in the lumen because here the surface of fascia is bathed by blood.

Gallie and Le Mesurier (1) emphasized the value of fascia as "a living suture" and this is what it is. Proper use of fascia as suture or supporting structure, however, requires some knowledge of its slippery qualities on the part of the surgeon. Knots tied in fascial strips, even secure knots, tend to become free unless fastened with sutures. Where sheets of fascia are used to bridge a defect in fascia or muscle, the graft should overlap the fascia or muscles to which it attached so as to provide a broad, stro surface of attachment.

Special care is required in suturing t ends of fascia grafts to host tissues, becau the fibers run in a longitudinal direction a sutures readily pull out in the direction the fibers. Overlapping of the fascia gra and retention with silk mattress sutures stainless steel are helpful in obtaining strong union. When fascia is used as a pat to support herniation, *it is advisable to u* two fascia grafts in apposition to one anoth and arranged so that the fibers of each gra are at right angles to those of the other. Th provides resistance to separation of the fibe in all directions, and the graft tissues are thin that adequate blood supply is readi provided by the host tissues.

Keeping in mind that fascia should b used only as a buried graft and the fact th it will split and tear in the direction of i fibers, the surgeon, for all practical purpose can use it as a living suture or supporti substance whenever the need arises. Fasc possesses considerable strength in the dire tion of its long fibers but these fibers readil separate and pull apart when stress an strain are exerted in a right-angle directio The strength of a sheet or patch of fasci bridging a defect is also dependent on th strength of the fibrous attachment which obtains, and allowance must be made fo this factor in all repairs.

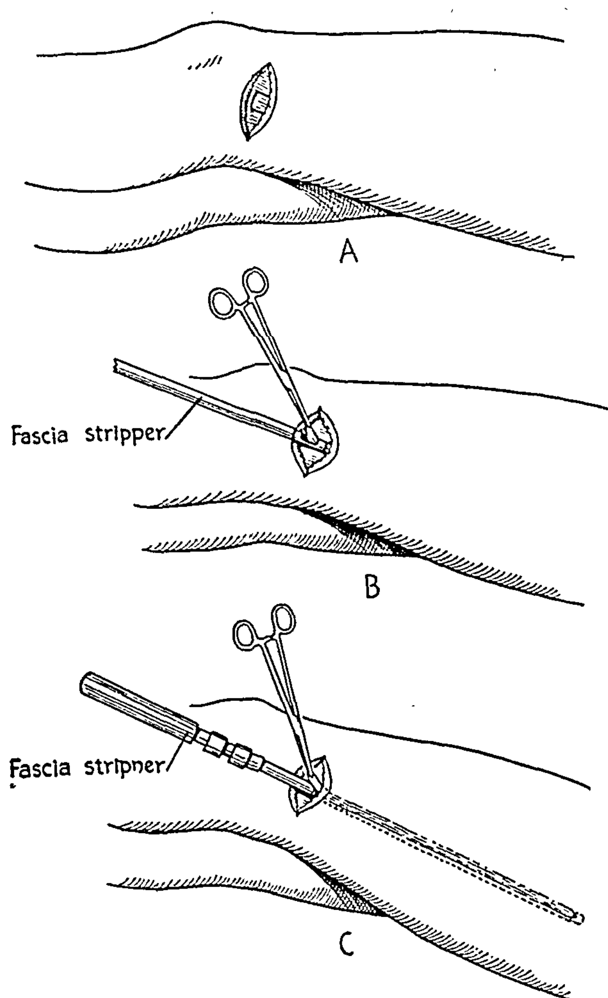


FIG. 117. Obtaining the fascia graft.

A. An incision has been made in the skin and subcutaneous tissue down to the fascia. Incisions have been made in the fascia.

B. The incised fascia has been brought through the opening in the end of the fascia stripper and is being held by the clamp.

C. The fascia stripper has been advanced the required distance and the upper end of the fascia is about to be incised by rotating the outer tube of the stripper.

From Principles and Practice of Plastic Surgery, Arthur Joseph Barsky. Baltimore: The Williams & Wilkins Co., 1950.

During the early part of this century surgeons undoubtedly used fascia in correcting conditions for which it was not suitable, but in this connection one must bear in mind that experience and skills are not equally distributed, and some surgeons use fascia more successfully than others. The quality and tensile strength of fascia also vary in different patients, and this is an important factor in obtaining successful repair with fascia grafts.

Herniation of the muscle at the donor site in the thigh sometimes occurs but this seems to disappear in 8 to 12 months after operation due to partial regeneration of the fascia, which fills in the gap and supports the muscles. The fact that defects resulting from the removal of fascia lata strips in the thigh are again covered with fascia probably accounts for the failure of Kondolean type operations for lymphedema of the leg. This is because the regenerated fascia again forms a barrier preventing drainage of the waterlogged subcutaneous tissues and skin into the venules and lymphatics of the muscle. It is expedient to remove the fascia in the calf and thigh widely in such patients to provide a more permanent circulatory exchange. This also applies to the removal of the deep fascia over the triceps and biceps muscles of the arm in lymphedema of the arm following radical breast amputation as advocated by the author.

Fascia grafts are extremely valuable as substitutes for tendon in certain locations. For instance, fascia may be employed to bridge gaps in the extensor tendons of the hands, whereas, on the contrary, it is usually better to use tendon grafts to replace the flexor tendons of the hand.

Fascia grafts are useful as substitutes or for strengthening of ligaments which have become weakened because of injuries. Thus, fascia grafts were used by Gallie and Le Mesurier for habitual dislocation of the patella, ununited fracture of the patella, and infantile paralysis of the shoulder in small children. They also reported the successful use of fascia grafts for visceroptosis, inguinal and ventral hernia,[1] for femoral hernia, and for dislocation of the shoulder.

Nicola (2) and others use ligament or

[1] In the author's experience diced-cartilage grafts are more satisfactory for recurrent hernia and large ventral hernia than fascia grafts (see Chapter 14, Clinical Use of Cartilage Grafts).

CHAPTER 26

Clinical Use of Fascia and Tendon Grafts

CLINICAL USE OF FASCIA GRAFTS

Undoubtedly, fascia grafts in the past were used in correcting conditions for which they were not suitable. For instance, fascia is a tissue which is not normally found on the body surface or on the exposed surfaces of body cavities such as the stomach, bladder, lumen of glands and so on. In these locations fascia, anatomically, is always protected by covering cells. Generally, therefore, fascia, like tendon, should be used only as a completely buried graft and not transplanted in locations where one surface of the graft is exposed. One is inclined to believe, however, that autogenous fascia grafts may again become popular for the repair of deficiencies in blood vessels instead of homogenous blood vessel segments and "tolerated foreign-body" pipes or wrappings. Fascia grafts used to repair blood vessels are not actually exposed in the lumen because here the surface of fascia is bathed by blood.

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Special care is required in suturing the ends of fascia grafts to host tissues, because the fibers run in a longitudinal direction and sutures readily pull out in the direction of the fibers. Overlapping of the fascia graft and retention with silk mattress sutures or stainless steel are helpful in obtaining a strong union. When fascia is used as a patch to support herniation, *it is advisable to use* two fascia grafts in apposition to one another and arranged so that the fibers of each graft are at right angles to those of the other. This provides resistance to separation of the fibers in all directions, and the graft tissues are so thin that adequate blood supply is readily provided by the host tissues.

Keeping in mind that fascia should be used only as a buried graft and the fact that it will split and tear in the direction of its fibers, the surgeon, for all practical purposes, can use it as a living suture or supporting substance whenever the need arises. Fascia possesses considerable strength in the direction of its long fibers but these fibers readily separate and pull apart when stress and strain are exerted in a right-angle direction. The strength of a sheet or patch of fascia bridging a defect is also dependent on the strength of the fibrous attachment which it obtains, and allowance must be made for this factor in all repairs.

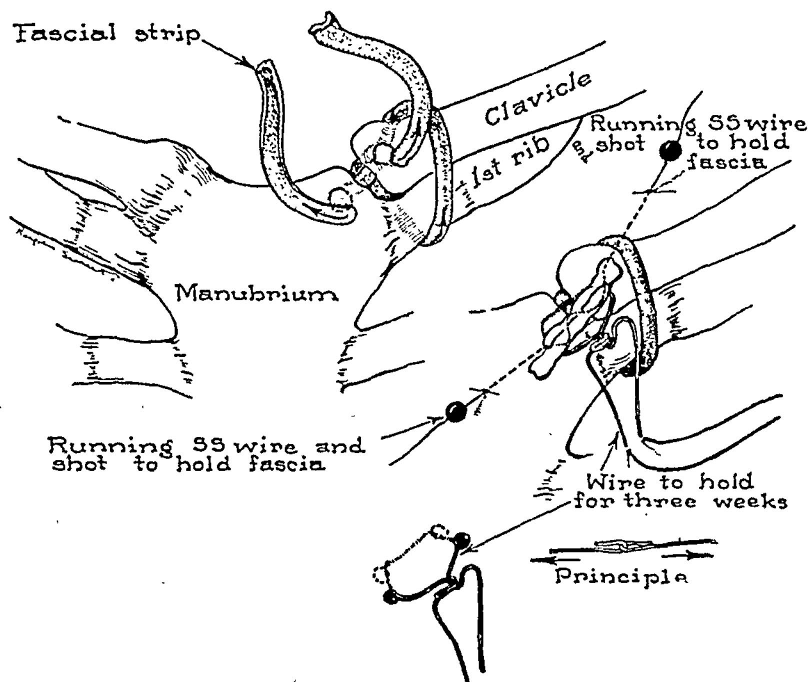


Fig. 119. Operation for dislocation of sternoclavicular joint. Fascia lata a foot long is threaded through two holes and around the first costal cartilage in the manner shown and fastened to itself in front with a removable stainless-steel wire fastened outside the skin with two shots. The joint is kept from dislocating while the fascial graft is healing by a No. 22 stainless-steel wire placed through the two holes and fastened to itself in such a way that it may be withdrawn in 3 weeks. On pulling each wire, both of which emerge through the skin, the bends straighten out and the wire can be withdrawn. This principle is applicable for bones elsewhere. The method has been carried out by the author with success. From Surgery of the Hand, Sterling Bunnell. Philadelphia: J. B. Lippincott Co., 1944.

ment to use the free fascia graft when local fascia in the operative area is available and can be employed satisfactorily.

In blood vessel surgery the preserved arterial homograft seems to have replaced the autogenous fascial tube, but techniques in surgery change about as frequently as styles in clothes (men's clothes). As previously stated, autogenous fascia may again become popular in vascular surgery.

CLINICAL USE OF TENDON GRAFTS

Tendon grafts have their most valuable clinical use as a substitute for tendon. When tendon grafts are used for this purpose, the slippery covering, paratenon, should always be included with the graft to allow for a normal gliding mechanism.

Tendon differs grossly from fascia not only in its thick rope-like form but also in its possession of a specialized and loosely adherent slippery covering, the paratenon. This paratenon permits the tendon to move to and fro in response to contraction and relaxation of its motor or muscle. Bunnell has described the elastic fibers which connect the paratenon and tendon. During relaxation these elastic fibers are coiled up like springs but on movement of the tendon they are stretched out, and at the limit of the stretch the paratenon is dragged to and fro to some extent along with the tendon.

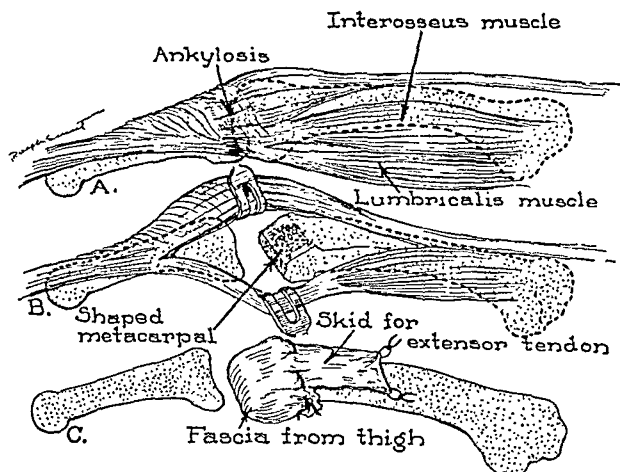


Fig. 120. Arthroplasty for ankylosis of proximal finger joint. Gives about 70° of motion. The prolongation proximally of the fascial hood is used in case the extensor tendon is found adherent to the bone. From Surgery of the Hand, Sterling Bunnell. Philadelphia: J. B. Lippincott Co., 1944.

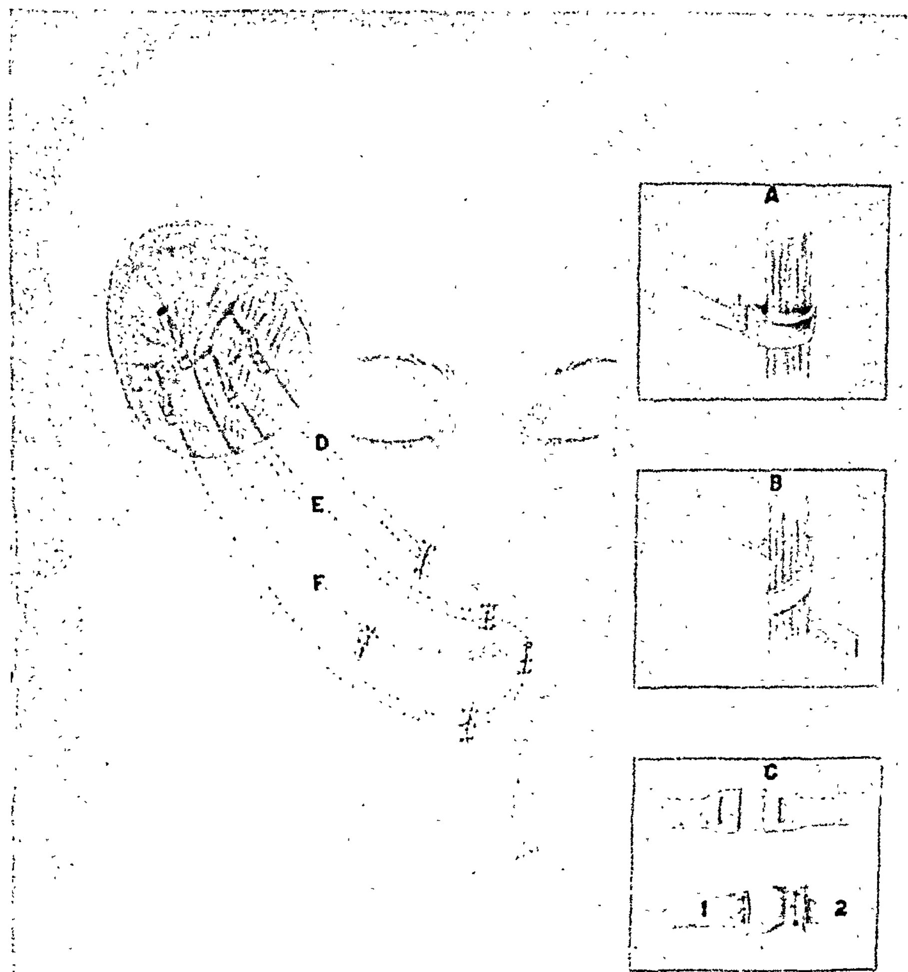


FIG. 118. Distribution and fixation of fascial strips for mechanical support of mouth and lower end of nose. *Insets*, *A*, fixation of single strip around a muscle bundle; *B*, continuous fascial strip around a muscle bundle; *C*, splicing fascial strips. Letters and numbers on the face of the illustration which are not explained in the legend are accounted for in the text. From Plastic and Reconstructive Surgery, Ferris Smith. Philadelphia: W. B. Saunders Co., 1950.

tendon for correcting recurrent dislocation of the shoulder, and the procedure is an established and accepted operation in orthopedic surgery because of its evident success.

Plastic surgeons employ fascia grafts to support the paralyzed side of a patient's face and to elevate a drooping eyelid by attaching the lid to the frontalis muscle.

Fascia was recommended by the late John Wheeler to fill out small depressions in the eyelids. It is quite useful where a small amount of filling is required to obtain normal facial contour. In cerebral spinal fluid leakage through fracture in the cribriform plate the open dural defect may be closed with a free fascia graft. This procedure was often used by the late Dr. Wells P. Eagleton of Newark, who was a pioneer in treating intracranial surgical infections from the sinuses and mastoid before the day of antibiotics.

One should bear in mind that tendon cut into thin strips may be employed in place of fascia. Dermis of the skin, which is available in great quantities and has considerable tensile strength, may also be used as a substitute for fascia grafts.

Obviously in surgery the simplest way is always the best way, and it is not good judg-

vessels anastomose with the severed ends of vessels in the paratenon and provide early circulation for the tendon graft, thus retaining the gliding mechanism. Tendon grafts denuded of paratenon also obtain early circulation through blood vessel anastomoses but they tend to become adherent to the surrounding connective tissues. In this connection one must remember that tendon normally has a blood supply through small vessels in its covering paratenon and epitenon (within tendon sheaths). Vessels in the epitenon within tendon sheaths are said to penetrate the tendon itself.

Possibly in some locations the vessels end in the tendon covering, and tissue fluid exuding from this capillary bed permeates the intercellular substance of the tendon. It is stated that capillaries actually penetrating the tendon from its slippery covering are present only (or largely) in locations where tendon is within a definite tendon sheath. The author has observed, however, that where a tendon graft is not within a tendon sheath, it develops a rich blood supply through blood vessels in its covering paratenon in about three days after transplantation. These vessels seem to penetrate the tendon substance and were probably present at the time of transplantation, since rich circulation was developed in this short period of time. It is possible, of course, that blood vessel sprouts penetrated the graft and anastomosed with graft vessels.

Wherever tendons turn a corner, a tendon sheath is usually provided, and this is supported by a fibrous tunnel and fibrous bands to prevent bowing of the tendon when the muscle contracts. The free or attached tendon transplants must be threaded through these tunnels to provide efficient function. When the tunnels are absent, substitutes should be provided in the form of fascia or tendon graft bands; for these serve as pulleys and also prevent bowing of the tendon away from the bone when the muscle contracts

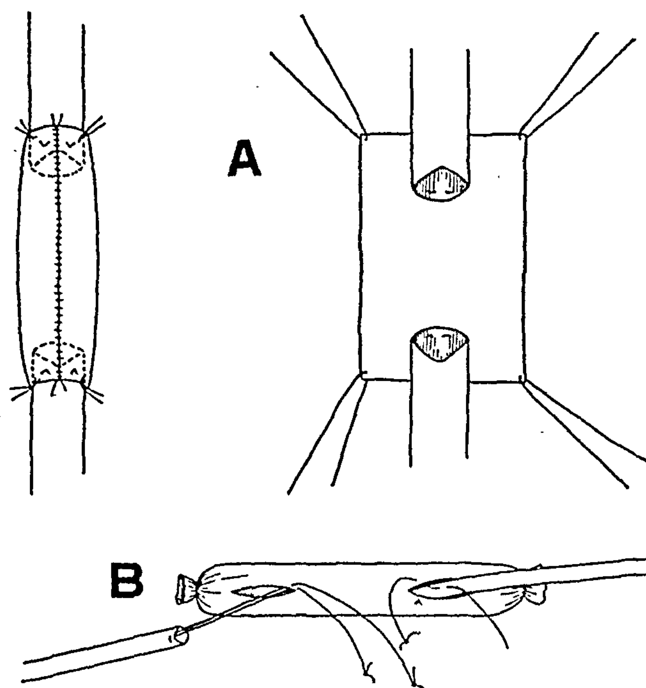


Fig. 124. A. Diagram to show the method of constructing a fascial tube between the ends of a resected ureter.

B. Diagram illustrating the method of fixing the ends of a resected ureter into a previously constructed tube of fascia. On the right, the ureter is being drawn through a slit in the tube. On the left, the wall of the ureter is being fixed to the tube at the slit. The slits in the fascial tube are drawn too large.

From The Transplantation of Tissues, H. Neuhof. New York: D. Appleton and Co., 1923.

Where tendons are severed from their connection with bone and shifted to another bony attachment together with their muscle, this transfer is, to some extent, in the nature of a pedicled flap. The tendon receives a limited blood supply from its muscle but the distal portions of the tendon must receive additional blood supply from the surrounding host tissues and from the new host bony or tendinous attachment. These pedicled flap tendon grafts should also be shifted with covering paratenon or epitenon to provide a free gliding mechanism for the tendon in its new location.

The tensile strength of a tendon graft like that of a fascia graft depends on the resistance of the healing scar which attaches the tendon to host tendon or to host bone. The

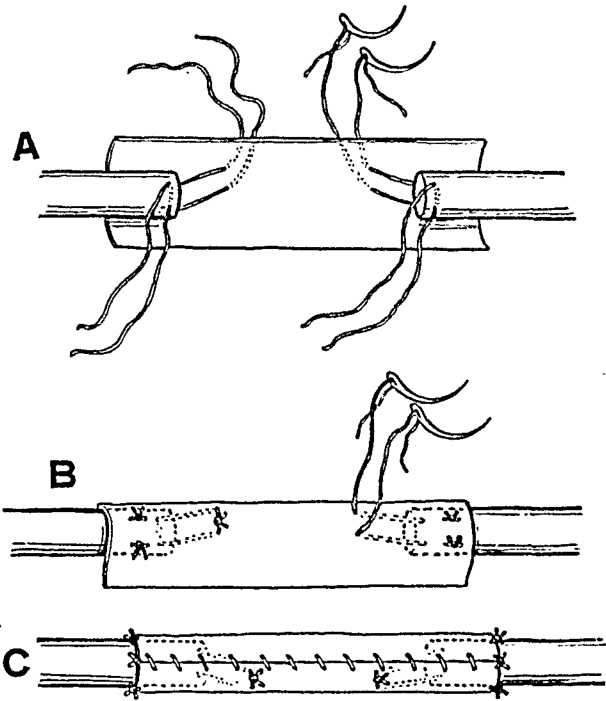


FIG. 121. A. The technique of fascial grafting for a tendon defect. The sheet of fascia is placed beneath the ends of the tendon. Two sutures are passed through each end of the tendon. The deep pair are being passed through the graft so that the ends of the tendon are drawn towards each other when these sutures are tied.

B. Posterior view to show the completion of the deep sutures and the attachment of tendon to graft by additional sutures.

C. The anterior traction sutures on the ends of the tendon are completed, the cuff of fascia sutured to the adjacent surface of tendon, and the margins of the graft are approximated by a continuous suture.

Neuhof in a similar way used a cuff of autogenous fascia to substitute for an absent segment of a large artery. Present-day clinicians in vascular surgery appear to have given this method up in favor of arterial homografts, foreign-body tubes and the like. Autogenous fascia has many obvious advantages when autogenous veins of sufficient size and strength cannot be obtained. Autogenous veins reinforced with a cuff of fascia are worth considering. The composite grafts could be reinforced by a perforated cylinder of tantalum on the principle of the perforated vitallium ear mold, or diced cartilage might be used between the fascia and the perforated tantalum cuff to provide rigid strength, which would withstand the force of arterial pulsations. (See Clinical Use of Cartilage Grafts, Chapter 14.)

From The Transplantation of Tissues, H. Neuhof. New York: D. Appleton & Co., 1923.

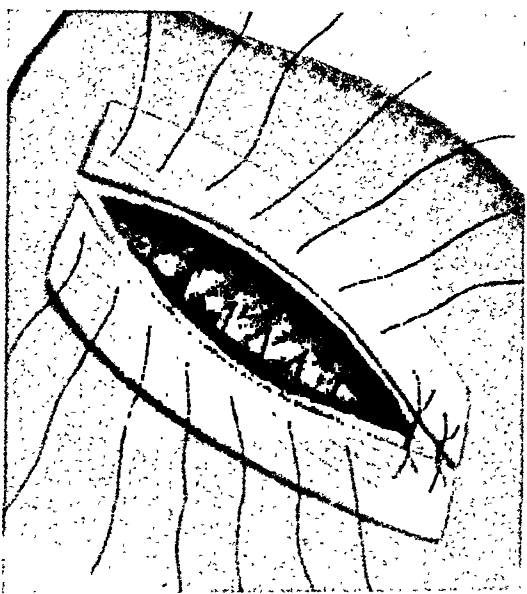


FIG. 122. Suture of a superficial wound of the liver with supporting strips of fascia (Kirschner). From The Transplantation of Tissues, H. Neuhof. New York: D. Appleton and Co., 1923.

This paratenon should always be included with a free tendon graft or with a tendon attached to its muscle to provide a gliding mechanism for the transplanted tendon. Fascia has a limited gliding mechanism because of its loose attachment to adjacent tissues, but this is not as specialized or efficient as paratenon.

The paratenon is also important for the early circulation in tendon grafts. Host blood

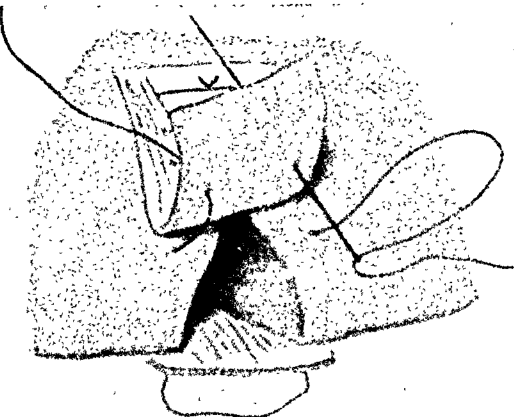


FIG. 123. Suture of a wedge-shaped wound of the liver with fascial reinforcement (Kirschner). From The Transplantation of Tissues, H. Neuhof. New York: D. Appleton and Co., 1923.

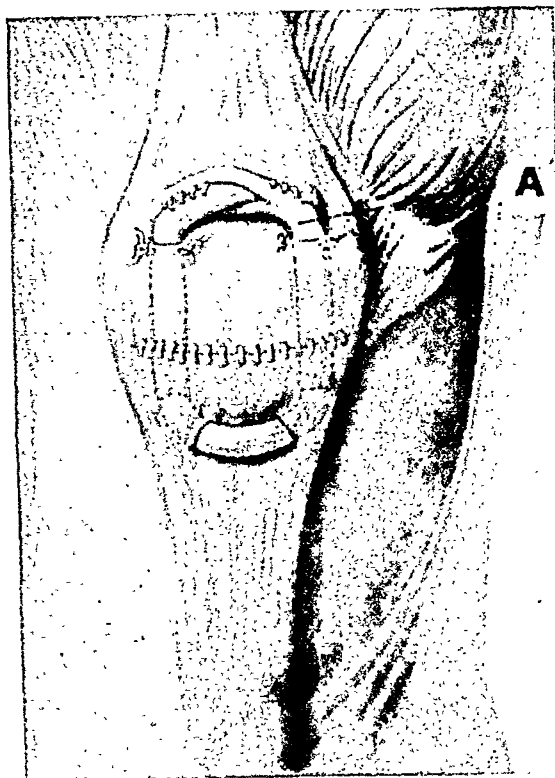


FIG. 126. Drawing of operation for repair of ununited fracture of patella in which one of the fragments is a mere flake of bone.

A. Heavy suture of fascia lata passed vertically through the patella and transversely through the ligamentum patellae. From The Transplantation of Fibrous Tissues in the Repair of Anatomical Defects, W. E. Gallie and A. B. LeMesurier. Brit. J. Surg. 12: No. 46, 1924.



FIG. 127. Drawing of operation for habitual dislocation of the patella.

A. Strand of fascia lata, half an inch wide, passed through holes in the patella and internal condyle and fastened with catgut. From The Transplantation of the Fibrous Tissues in the Repair of Anatomical Defects, W. E. Gallie and A. B. LeMesurier. Brit. J. Surg., 12: No. 46, 1924.

tendon will form a channel in the fat and permit a surprisingly good gliding mechanism in the absence of paratenon.

Although tendon grafts are utilized mainly as a substitute for deficiency in tendons and as pulleys about tendons where the latter must turn a corner, they also have other uses. Tendon grafts in their complete thickness or in strips serve as strong living suture material and in this connection may act as substitutes for strips of fascia or dermis of the skin. The available supply of autogenous tendon, however, is often limited to the palmaris longus and the long extensors of the foot.

In patients in whom the flexor sublimis and profundus have been severed for some time, it may be wise to sacrifice the sublimis and use it as a graft for the profundus. In these patients the proximal end of the sublimis should always be attached to the profundus to give the latter greater pulling power and to prevent adhesion between the sublimis and other tendons in the vicinity.

In spite of numerous articles on the subject of preserved homogenous and heterogenous tendon grafts in humans *only the use of autogenous grafts is advised at this time.* The surgeon will have trouble enough with autogenous grafts, especially for the flexors of the hand, without complicating the procedure by the use of foreign grafts, which

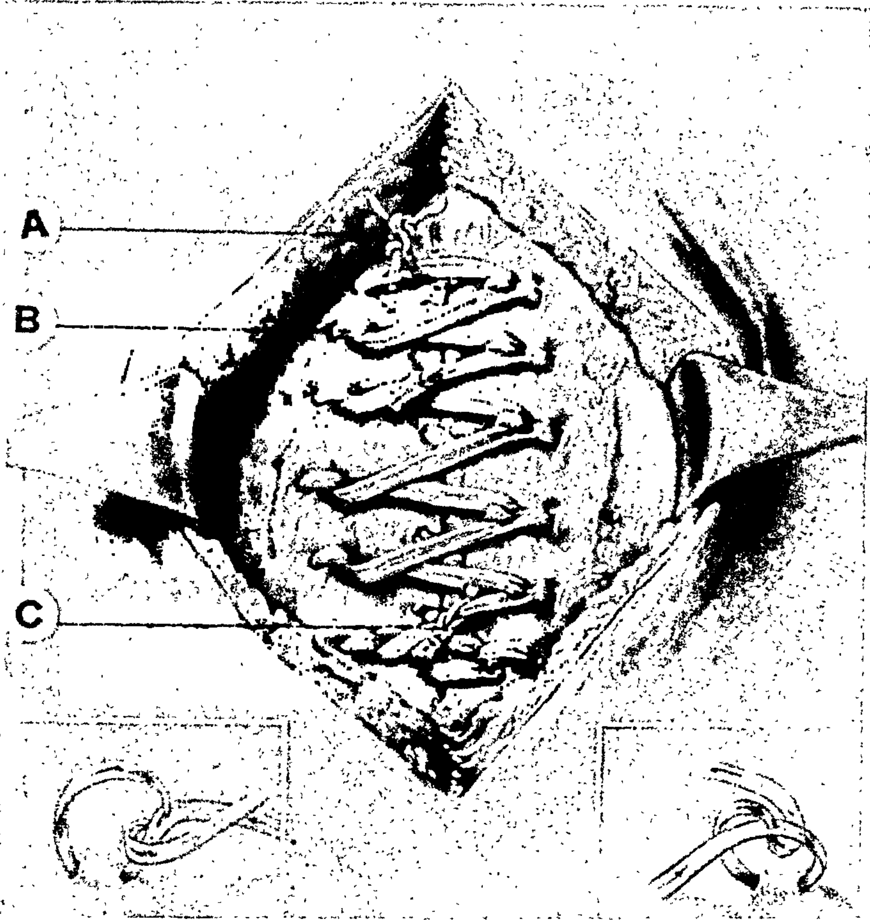


Fig. 125. Drawing of the repair of ventral hernia. Insertion of second row of sutures. The needle takes deep bites of the aponeurosis at some distance back from the edges of the opening.

a, A join between two sutures; *b*, Lock-stitch inserted to prevent slipping; *c*, Triple knot which terminates the suture. It is transfixed with a ligature of fine silk or catgut.

The inserts in the lower corners show methods of making the lock-stitch.

From The Transplantation of the Fibrous Tissues in the Repair of Anatomical Defects, W. E. Gallie and A. B. LeMesurier. Brit. J. Surg. 12: No. 46, 1924.

surgeon is therefore in considerable dilemma in regard to when he should allow his patient to begin active movement of the graft through action from its attached muscle. Early movement, which favors free gliding, may separate the new scar formation and may rupture new blood vessels which have formed anastomoses with vessels in the paratenon.

Bunnell (3), in his excellent book, and others have described the optimum time for active movement of tendon grafts in various locations, and the reader is referred to texts on this subject for detailed account of proper management.

It is important always to cover exposed tendons which have their paratenon removed, with fat or slippery paratenon rather than bring them into direct contact with the dermis of a skin graft. In the latter case the skin will become adherent to the tendon, and movement is restricted, since the tendon must drag the overlying skin to and fro as the muscle contracts. Exposed tendons with absent paratenon should be covered by fat and overlying skin. Early movement of the

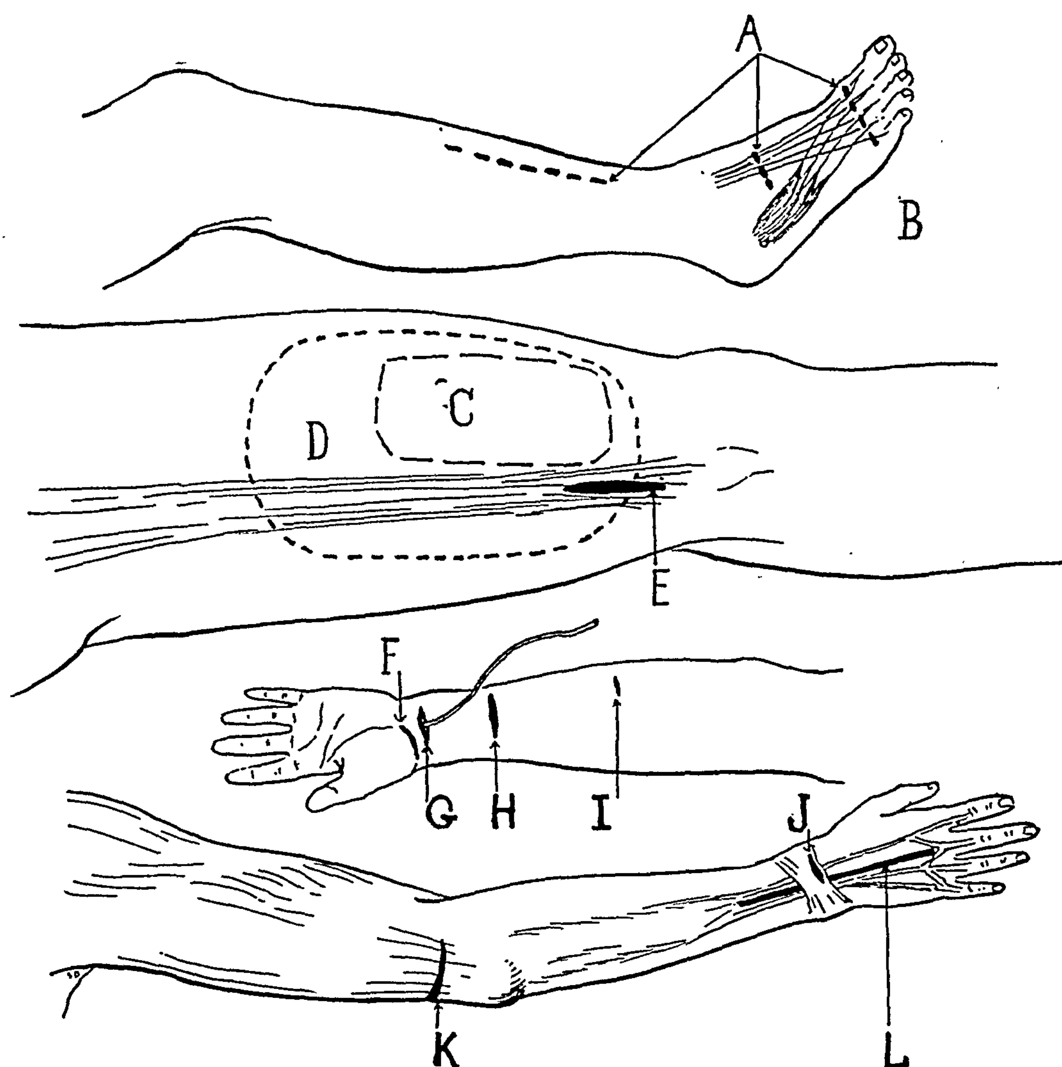


FIG. 129. Sources of grafts, with incisions through which they may be reached and for other purposes.

A. Extensor digitorum communis of the four small toes may be removed through the three incisions (dotted).

B. Extensor brevis which will continue to provide extension of the toes except the fifth.

C. Site for obtaining this deep fascia for a gliding surface to place under tendons.

D. Dotted outline of site of a thin layer of paratenon directly overlying the deep fascia used as gliding material for tendons.

E. Incision for obtaining with a fascial stripper a ribbon of fascia lata as indicated.

F. Incision for approach to the tuberosity of the scaphoid.

G. Incision for approach to the lunate.

H. Transverse incision through which the tendons of the flexor sublimis are withdrawn for grafts.

I and G. Two short incisions for removal of the tendon of the palmaris longus.

J. Incision for approach to the dorsal end of the scaphoid to place a bone-graft peg.

K. Incision for obtaining the layer of paratenon for gliding overlying the triceps tendon.

L. An extensor communis tendon may be spared for a graft. The juncturae tendinum will extend the finger.

From Surgery of the Hand, Sterling Bunnell. Philadelphia: J. B. Lippincott Co., 1944.

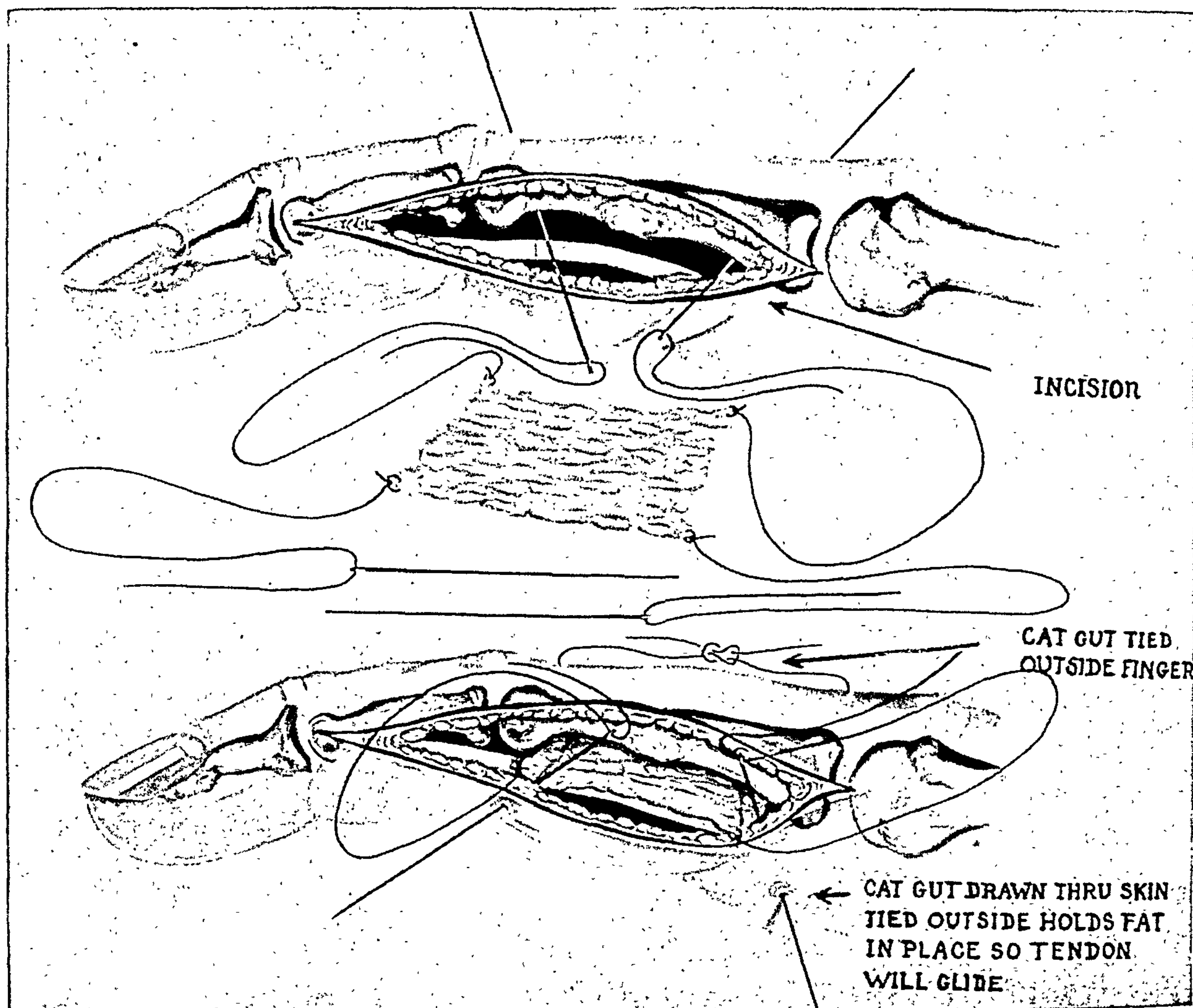


FIG. 128. After freeing a tendon from bone or scar, reattachment is lessened if a thin layer of slippery deep fascia or specialized paratenon is interposed.

Drawing shows an easy method of placing such a filmy graft. The No. 000 catgut is passed out through the skin and tied to itself on the outside of the finger.

From Surgery of the Hand, Sterling Bunnell. Philadelphia: J. B. Lippincott Co., 1944.

give rise to greater host tissue reaction associated with more fibrous adhesions.

The available evidence indicates that free autogenous grafts of both fascia and tendon survive as such in the human and only fresh autografts should be used for clinical purposes.

QUESTIONNAIRE OPINION REGARDING BEHAVIOR OF FREE FASCIA AND TENDON GRAFTS IN HUMANS

A questionnaire form was sent to 50 recognized orthopedic and plastic surgeons, asking for their opinions regarding the behavior of fascia and tendon grafts. This form, which was similar to the questionnaire on bone graft, did not meet with enthusiastic support. The author found it difficult to obtain opinions even from old professional friends when they were aware that these views were to be published. This is rather surprising, since almost everyone who was solicited submitted his convictions regarding the clinical and experimental behavior of bone grafts in humans very promptly.

Apparently many clinicians are not suffi-

ciently certain at this time (April 1954) to make definite statements concerning the behavior of free fascia and tendon grafts in the human. The wide experience and caliber of the surgeons answering the questionnaire, however, have induced the author to quote their opinions in order to establish reflections on the fate and clinical use of tendon and fascia grafts in the year 1954.

All men answering the questionnaire had the impression that under favorable conditions of transplantation and use both autogenous fascia and autogenous tendon grafts, when exposed at a secondary operation, appeared grossly like fascia and tendon. Most of these surgeons believe that the grafts survived as such and did not become counterfeit structures fabricated by the host tissues.

Sterling Bunnell holds that healing in severed tendons occurs first by connective-tissue proliferation from paratenon, epitenon, endotenon, and surrounding connective tissue, followed by active growth of the tendon cells. He further believes that the cells in tendon and fascia grafts tend to survive as such although in thick tendon grafts the centrally-located cells may fail to live because of nutritional difficulties. Bunnell has had no experience with fresh or preserved homogenous grafts or with preserved heterogenous grafts.

Gallie and Le Mesurier of Toronto believe that cut tendons form definite healing through the activity of tendon cells. The cells in autogenous fascia grafts live unchanged if transferred immediately, and are not allowed to dry out. The cells in autogenous tendon grafts die if the transplant is thick. The tendon cells live, however, if the transplant is thin (like the palmaris longus), or if thick *autogenous grafts are split into thin strips.* The warning that autogenous tendon grafts may die if thick, is based on Gallie and Le Mesurier's experience with some patients on whom Nicola operations had been done for recurring dislocation of the shoulder, and with other patients in whom new crucial ligaments of the knee had been made. When these joints were opened, a few years later, no sign of the transplanted tendons could be found. They believe that the cells in fresh homogenous fascia and tendon grafts die and the graft structure is absorbed. Preserved homogenous and heterogenous fascia and tendon grafts, with dead cells, are absorbed after transplantation. Gallie and Le Mesurier consider only autogenous fascia and tendon grafts to be of clinical value.

It is Toufick Nicola's belief that healing of

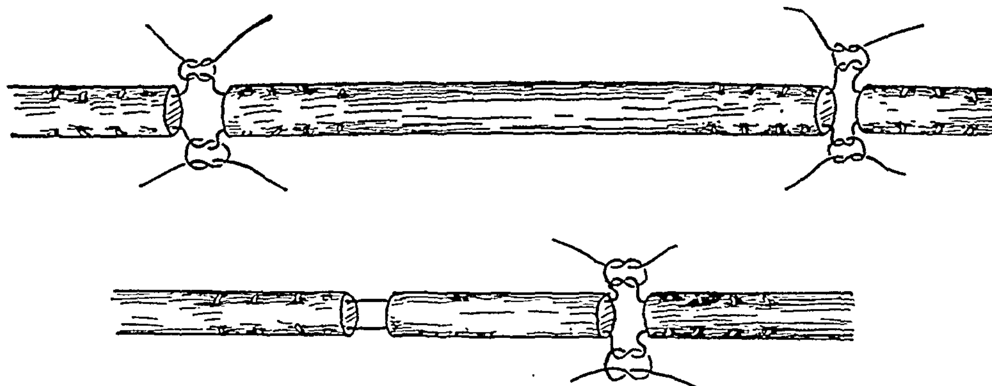


FIG. 132. Method of suturing a free tendon graft to bridge a gap in a tendon.
Below. Method of bridging a short gap. In the latter case, after the sutures have been drawn up and tied, each of the two ends of the graft is lightly attached to the tendon end by a single stitch of fine catgut merely to maintain approximation. (Courtesy, J. Bone & Joint Surg., 10: 11, 1928.)
From Surgery of the Hand, Sterling Bunnell. Philadelphia: J. B. Lippincott Co., 1944.

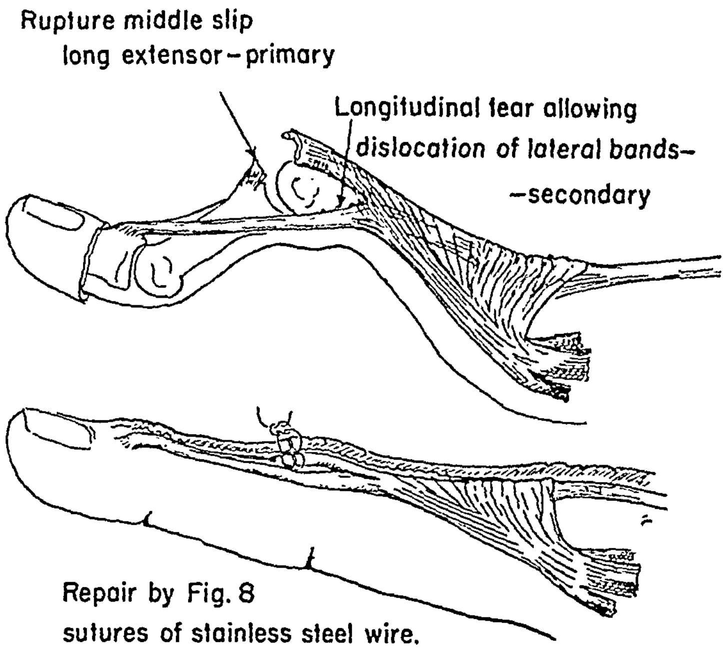


Fig. 130. The *"boutonnière"* deformity is caused by rupture of the central extensor tendon slip at the middle joint and a subsequent tear of the aponeurosis. The lateral bands then flex the middle joint and extend the distal joint.

A simple figure-of-eight removable stitch of stainless-steel wire, together with splinting, is sufficient to cure a recent patient. In a long-standing case, the slit also should be closed by a removable figure-of-eight suture through the skin and tendon. (Courtesy, J. Bone & Joint Surg., 24: 20, 1942.)

From Surgery of the Hand, Sterling Bunnell. Philadelphia: J. B. Lippincott Co., 1944.

Fig. 131. Method of attaching tendon to bone, using removable stainless-steel wire as used in the pulley operation for opposition of the thumb or the *tendon* T operation. There is tendon-bone contact. The wire penetrates the digit or limb and is tied over a button outside the skin. The suture in three weeks is withdrawn by the pull-out wire.

From Surgery of the Hand, Sterling Bunnell. Philadelphia: J. B. Lippincott Co., 1944.

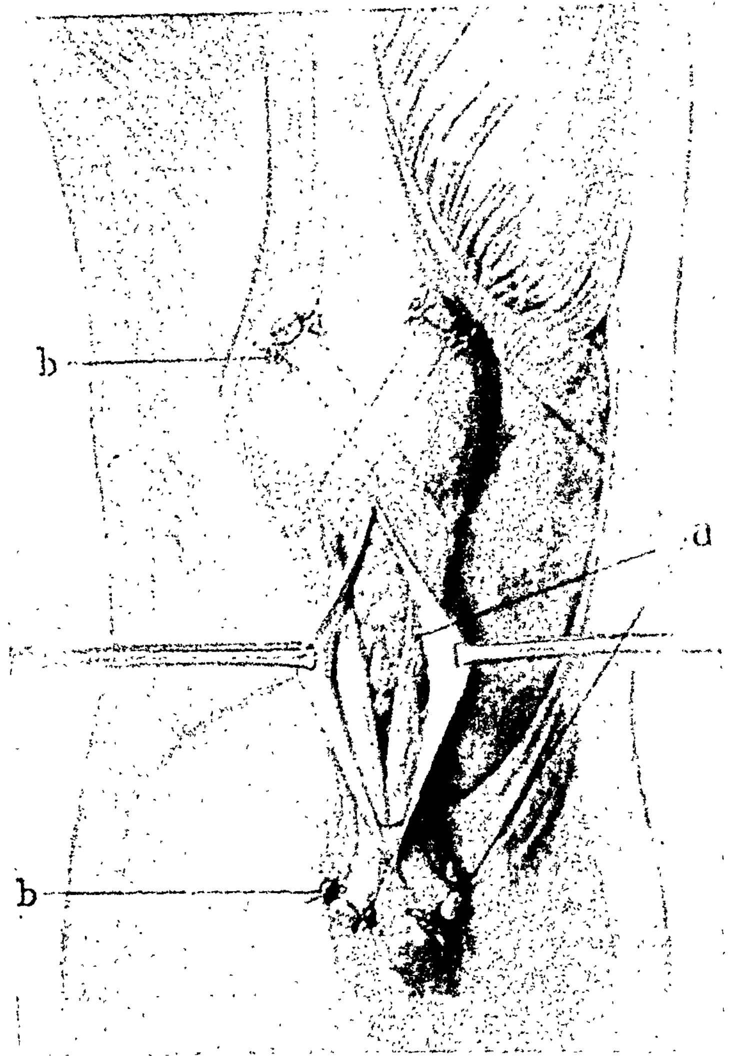


FIG. 134. Drawing of operation for the repair of ruptured ligamentum patellae. *a*, Segment of tendo Achillis drawn through holes in patella and tibia. *b*, Kangaroo sutures which fasten the transplant in place until firm healing occurs. From The Transplantation of the Fibrous Tissues in the Repair of Anatomical Defects, W. E. Gallie and A. B. LeMesurier. Brit. J. Surg. 12: No. 46, 1924.

the tendon is subjected to use after healing has occurred (in 3 to 4 weeks). Otherwise they may not survive and the graft becomes scar. Autogenous fascia grafts behave in the same way as autogenous tendon grafts. Mason has had no experience with fresh or preserved homogenous tendon and fascia grafts, or with preserved heterogenous grafts. He believes that autogenous tendon grafts must be kept under their normal tension and direction of pull if they are to survive as such.

Darrel T. Shaw of Cleveland holds that healing of cut tendons occurs through the activity of connective-tissue cells from the surrounding host tissues and the sheath, and also through proliferation of tendon cells. His clinical experience with autogenous tendon grafts has been satisfactory if the slippery paratenon is present, and the same with autogenous fascia grafts from a clinical standpoint. Shaw does not express an opinion concerning the survival of the cells in autogenous tendon and fascia grafts. He has not used fresh or preserved homogenous or preserved heterogenous grafts of fascia or tendon.

Milton T. Edgerton of Johns Hopkins Hospital, Baltimore, states that in humans he has had success only when fresh autogenous tendon or fascia grafts are employed. With tendon grafts the clinical results are much more satisfactory if the graft is transplanted together with the tendon sheath or slippery paratenon. When some of the hands are reopened years after grafting, the tendons grossly resemble the original graft, and his microscopic sections tend to confirm this

cut tendons comes first from the epitenon, tendon sheath, paratenon, and endotenon. Proliferation of tendon cells begins after the fourth week. The cells in autogenous tendon grafts are at first nourished by the surrounding lymph and tissue juices. The center becomes necrotic but after vascularization occurs the dead centrally-located cells are replaced by new tendon cells and fibers (presumably through activity of the surviving tendon cells in the graft). Fresh homogenous tendon grafts in contact with tendon are replaced by creeping substitution of live tendon tissue, and fresh homogenous fascia grafts show the same behavior. Fresh homogenous tendon grafts are not as satisfactory as autogenous grafts, because the former become adherent due to foreign-body reaction. Nicola has had no experience with preserved homogenous or preserved heterogenous fascia and tendon grafts.

As viewed by Michael L. Mason, the initial healing of cut tendons is a formation of callus from the surrounding host tissues. Later (from 7 to 10 days) the tendon cells begin to take part and, as time goes on, in successful healing the tendon cells assume more and more of the role in repair. Mason has the impression that the tendon cells in autogenous tendon grafts survive as such if

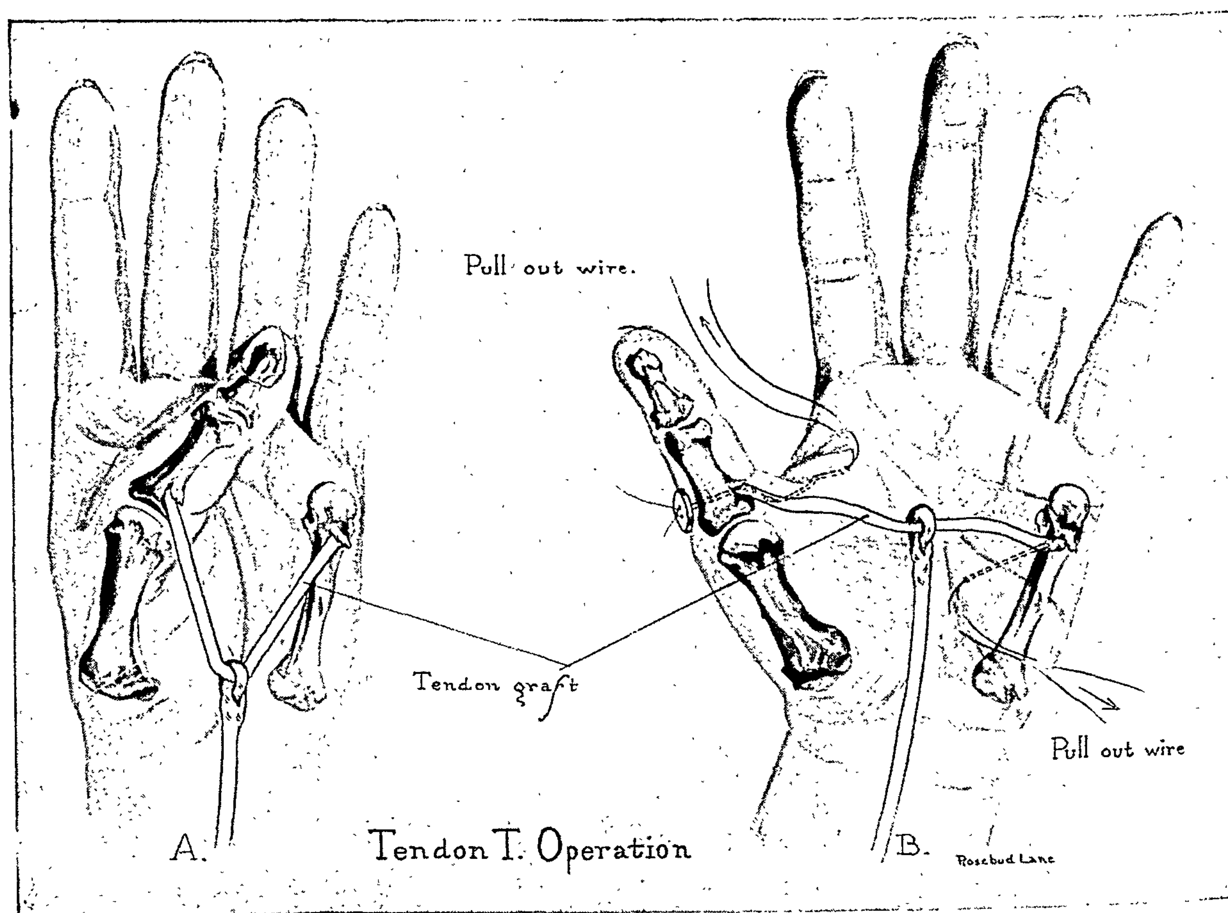


Fig. 133. In the tendon T operation to restore adduction to the thumb and curvature to carpal and metacarpal arches, a tendon graft spans the distance between the little finger metacarpal and the adductor insertion in the proximal phalanx of the thumb. A long flexor tendon of the forearm (a sublimis or the palmaris longus prolonged by a strip of its palmar fascia) is looped over its center to form the T. This, when in action, changes to a Y, adducting the thumb and curving the arches.

From Surgery of the Hand, Sterling Bunnell. Philadelphia: J. B. Lippincott Co., 1944.

PART V

Muscle

impression. Edgerton has had no experience with homogenous or heterogenous tendon or fascia grafts in the human.

Herbert Conway of Cornell Medical College uses only autogenous tendon and fascia grafts but has not studied their behavior experimentally.

William Frachelton of Milwaukee also uses only autogenous tendon and fascia grafts. Autogenous tendon grafts are successful and, when transplanted with paratenon tissue, will glide even in channels. Preserved homogenous tendon and fascia grafts are replaced, he believes, by fibrous tissue and the tendons become adherent to the surrounding host tissues.

The author strongly concurs in the opinions of those advocating the sole use of autogenous tendon and fascia grafts. Homogenous and heterogenous grafts give rise to a more severe degree of host tissue reaction, associated with fibrous tissue adhesions. These adhesions tend to prevent or restrict the free movement of tendon grafts, and to delay healing in both tendon and fascia grafts.

On the basis of his own experimental work the author believes that the cells in human autogenous tendon and fascia grafts survive as such and are not replaced by host tissue cells. The blood vessels in the grafts also survive and circulation is established through end-to-end anastomosis between host and graft blood vessels (at about 3 to 4 days).

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CHAPTER 27

Structure of Skeletal Muscle

It seems appropriate to deal with muscle grafts after discussing tendon and fascia grafts because skeletal muscle is the motor which moves tendons, and skeletal muscle groups are covered with and supported by dense fascia. The three tissues—skeletal muscle, tendon, and fascia—are closely associated to produce movement in the jointed bones of the body and for other structures such as the eyes, face, and ears.

The fact that free muscle grafts are of no clinical value at this time, except to stop slow bleeding in surgical procedures, is no reason whatever to omit or shorten the chapter on this important tissue. Presentation of the known facts regarding skeletal muscle will serve as a basis for future experimental work, and one cannot preclude the possibility that successful free muscle grafting will some day be accomplished.

ACTIVITIES OF MUSCLE

Muscle is unique among mammalian tissues in that its activity can be seen and appreciated with the naked eye. All other direct cell activities can be observed only by means of high magnification.

The body without skeletal muscle movement would seem completely inert aside from the pulsation of the heart, and this would soon stop due to paralysis of respiration. The contracture and relaxation of skeletal muscle *make the human come alive* through functional movements of the limbs and body.

Subtle personality differences in individuals are imparted by movements of the eyes, the facial muscles, the control of speech, and the tilt of the head. These muscular movements in symphonic harmony have long been developed into a refined art to beguile, persuade, or force the desires of one human individual on another and are used to advantage during courtship. All lovers of the woods have noted the antics of the cock grouse to impress his mate, and the noisy courting methods of the amphibian frog, which are sometimes irritating to those who live near a pond.

In the human female soft curves are provided by a layer of fat, which softens the sharp outline of skeletal muscles, whereas in men a definite contour of skeletal muscles is both respected and admired.

Occasionally one sees cases of congenital lipodystrophy in girls in whom the fatty layer beneath the skin is absent and sharp muscular outline is apparent in the face, arms, and chest. In hemiatrophy of the face and more generalized scleroderma, the muscles in the involved area may be greatly reduced in size. On the other hand, in localized gigantism individual skeletal muscles or groups of muscles may be hypertrophied.

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flexion and its rôle in locomotion in terms that are acceptable today. At the Olympic games in Ancient Greece good muscular development was appreciated, and it was portrayed in statuary that has never been equaled. During the Renaissance the artist desired above all else to understand the mechanism of the human body in motion. The great master Vesalius one time exclaimed: "As for those painters and sculptors who flocked about me at my dissections I never allowed myself to get worked up about them to the point of feeling that I was less favored than those men, for all their superior airs."

Galvani, about 1791, noticed that fresh frog's legs hanging on copper hooks from an iron rack developed spasms when they touched iron. Thus was born the conception of animal electricity, elaborated more accurately by Galvani's gifted contemporary Volta (1).

Certainly, mobility is usually an attribute of living things. It may be noted in movements of cells, parts of cells or of material within cells. Motion of some sort seems to be universal in the physical world.

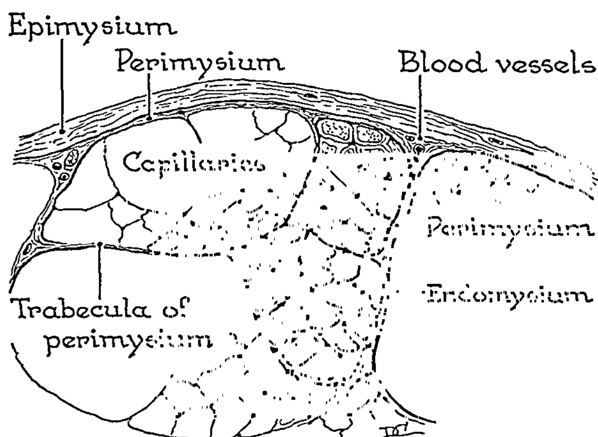


FIG. 135. Diagram of a cross section of a muscle. This shows the connective-tissue epimysium that surrounds the muscle. The partitions of perimysium that divide the muscle into bundles and the delicate connective-tissue endomysium that extends between the individual fibers in the bundles and carries the capillaries may also be seen. From Histology, Arthur Worth Ham. Philadelphia: J. B. Lippincott Co., 1950.

DEVELOPMENT AND STRUCTURE OF MUSCLE

Muscle develops from the mesoderm in the very early embryo (2), from rectangular blocks arranged in a row along each side of the neural tube. Each segment is innervated by its appropriate segmental nerve, and it is generally accepted that the portion of the segment which migrates to its normal locational site and differentiates into muscle, carries its nerve supply with it. Consequently, the origin of muscles in the adult can often be inferred from a consideration of their nerve supply.

Mesodermal cells in the central part of the rectangular blocks, called *myotomes*, give rise to the complicated and specialized muscle cells through the activity of an unknown organizer. Actual stages in the development of cross striations in the cytoplasm have been described by Speidel (3) in living tadpoles. The nuclei divide rapidly, cross striations appear in the cytoplasm, and the nuclei take up a peripheral position.

In cardiac muscle, according to Goss (4), and presumably in skeletal muscles, myofibrils and cross striation are not developed until a few hours after contractile activity is well established.

Muscle resembles fatty tissue in that it is composed largely of cellular elements with a relatively small amount of non-living intercellular fluid or gel. Like fatty tissue also, the individual muscle cells and cell groups are supported by a connective-tissue stroma which consists of parenchymal fibroblasts surrounded by their own collagenous intercellular substance. This connective-tissue stroma is condensed around muscle groups in the form of rather dense fascia.

The connective-tissue stroma is designated according to its location as: endomysium, which surrounds the individual muscle cells; perimysium, which surrounds groups of cells; and epimysium, which serves as a covering for the whole muscle. Blood vessels and

nerves are found in all three connective-tissue stromas including the delicate endomysium, by means of which the small vessels and nerves supply the individual muscle cells in the same way as individual fat cells are innervated and vascularized. The presence of actual lymphatic vessels within the stroma of individual skeletal muscle cells has not been established. It is stated that lymphatic vessels are present between the individual muscles and muscle groups. These vessels are present between the muscle cells in cardiac muscle because of the greater need for rapid exchange of lymph due to the constant activity of the heart.

The fibroblast cell in the stroma of free autogenous muscle grafts survives transplantation and is active in the replacement of the degenerating muscle cells in the graft. The fibroblast cell in the stroma of autogenous fat grafts also survives transplantation and is active in fibrous tissue replacement in regions where the fat cells have failed to survive.[1] Thus, in many ways fatty tissue and muscular tissue are quite similar in arrangement. An important difference, however, is the fact that probably all skeletal muscle cells die after free transplantation, whereas about 50 per cent of the fat cells in a free graft survive as living cells.

THE SKELETAL MUSCLE CELL

The skeletal muscle cell differs from all other ordinary tissue cells in that it is multinucleated like the giant cell. Smooth muscle cells have a single nucleus, and the cells in cardiac muscle do not exist as separate entities but instead are all joined together in such a way that they form a protoplasmic network. The nuclei of cardiac muscle cells or fibers tend to be disposed in the middle parts of the cells, whereas those in skeletal muscle cells are located in the periphery (5).

The skeletal muscle cell, excepting for the nerve cell, is the longest in the body. According to Ham, it may measure from 1 to 40 mm. in length. In the frog it may extend from its origin to the insertion of a muscle.

The skeletal muscle cell is enclosed by a thin apparently structureless membrane called the sarcolemma (cell membrane), much as a long sausage is covered with skin.

The cytoplasm or sarcoplasm of striated muscle fibers consists of longitudinally arranged fibers called myofibrils, which are cross striated. Electron photomicrographs reveal the fact that each myofibril is in reality a bundle of much smaller filaments like the fibrils in a collagenous fiber.

The property of contractility, which is a property of protoplasm, is brought to a high state of development in muscular tissue. The reader interested in the somewhat complicated processes involved during the contracture and relaxation of muscle fibers is referred to a text-book on histology.

As viewed by Szent-Györgyi (6) the contractile substance is a protein complex, actinmyosin. Neither this, nor either of its components, actin or myosin, alone shows any sign of contractility. What makes it work is *adenosine triphosphate*, which he calls ATP.[2] This, in his opinion, is not only the sole immediate source of the energy of muscular contraction, but it also dominates the whole physical state of muscle; without it muscle is inactive. ATP is absent in muscle showing rigor mortis.

According to Cowdry (7), actin appears to have the remarkable capacity of undergoing a reversible change from globular to the fibrous state and vice versa. This change is at least partly conditioned by the pH and ionic strength of the medium. At pH 6.5 the filaments of actin are many microns in length; while at pH 5.7 the length is decreased, but the width is unchanged. The

[1] Observations on autogenous human muscle and fat grafts by the author.

[2] ATP is probably absent in free muscle grafts a short time after transplantation. The complete severance of the blood supply in free grafts is an important factor, causing rapid degeneration and death of the muscle cells.

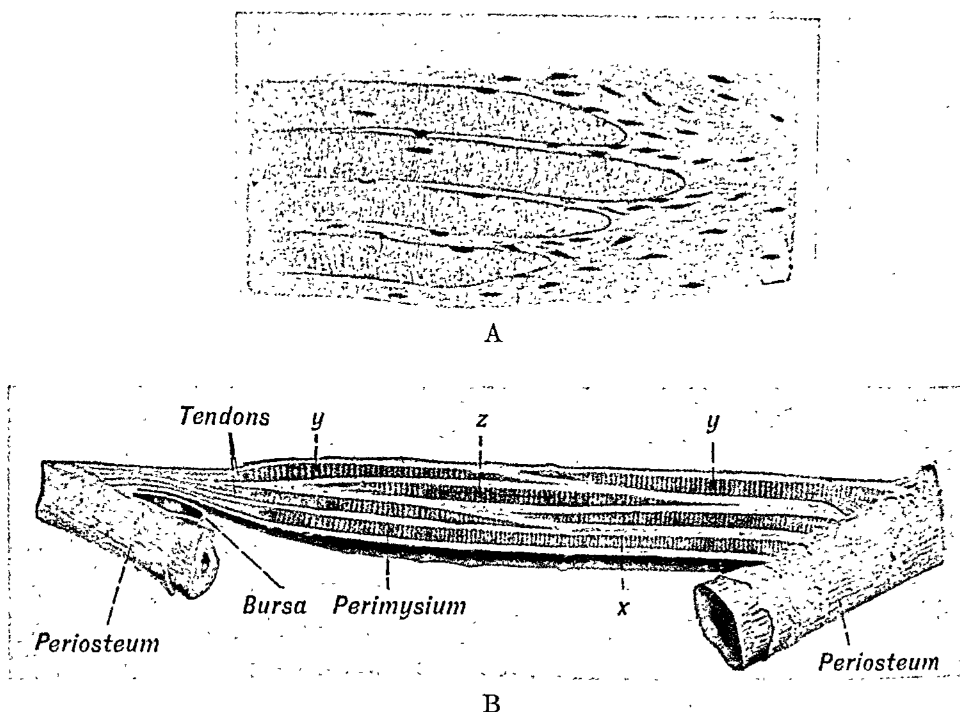


FIG. 136. A. The connection between the ends of the striated muscle fibers and a tendon. B. Diagram showing connections between a muscle and the bones to which it is attached. Muscle fiber, x, begins and ends in tendons attached to the bones; y, terminates at one end in the muscle z, terminates at both ends in the muscle.
From A Textbook of Histology, 6th ed., Alexander A. Maximow and William Bloom. Philadelphia & London: W. B. Saunders Co., 1949.

filaments break up into short rods at a lower pH.

The nuclei of striated muscle fibers are rounded or elongated in the direction of the long axis of the cell. In long muscle cells the nuclei may number several hundred and they are located at the periphery immediately beneath the sarcolemma.

MAINTENANCE AND REGENERATION

Maintenance

Striated muscle fibers cannot reproduce themselves, according to Ham (8). Hence an individual is born with all the striated muscle fibers he will ever possess. The growth of muscle that occurs in postnatal life is due to the individual fibers of muscle becoming larger. Exercise will promote the hypertrophy of the fibers of any given muscle.

Le Gros Clark (9) also states that it is probable that the full number of individual muscle fibers is acquired quite early in embryonic development although it is difficult to obtain final and conclusive evidence on this point. Adult human and rat skeletal muscle have been cultivated *in vitro* with the formation of myoblastic elements leading to regeneration and growth (10). The fact that myoblasts can become differentiated into cross-striated muscle cells under these conditions shows that the process is independent of nervous or other extraneous influence in developing embryos. From the cut ends of adult human striated muscle cells in tissue culture, protoplasmic buds grow out as in regenerating muscle fibers. These buds develop transverse striations, but before they do so they may become detached and undergo rhythmical contractions. The latter are variable in rate, being sometimes as rapid as 120 a minute, and occasionally they are also seen in well-developed

muscle fibers with cross striations. Clark believes that these observations are of considerable significance insofar as they demonstrate that the contractile properties of skeletal muscle *can develop quite independently of any nervous connections.*

In the author's opinion the fact that adult human skeleton muscle cells grow in tissue culture and undergo rhythmic contractures in the absence of a nerve stimulation is both impressive and suggestive. In the human body, however, when the motor nerve to a skeletal muscle is severed, the muscle becomes atrophied and eventually fails to contract in response to electrical stimuli.

The individual muscle cells with severed motor nerve become much swollen, and some may be replaced by fibrous tissue, but muscle cells with muscle structure continue to remain as such long after the motor nerve has been severed *provided the blood supply is intact.* Thus, the immediate and vital factor for the survival of muscle is maintenance of supply for necessary materials and elimination of waste products. Muscle cells and thin sheets of cells probably survive in tissue culture because the surrounding liquid can permeate the cell membranes and provide adequate exchange. Perhaps single cells or thin sheets of cells could survive in human tissues after free transplantation, whereas the usual free muscle transplants, which are composed of many layers of cells, are known to die after transfer. If it were possible to provide adequate immediate circulation for the cells in free muscle grafts and supply them with their normal pattern of nerve impulses, it is possible that muscle grafts would survive after transfer to the host. At any rate the solution is not more difficult than that of successfully transplanting a living homograft cell unprotected by cartilage or corneal matrix. There are many investigators working on the homograft problem.

Persistence of normal striated muscle fibers in the body is dependent on their continual use. Muscles which are not expanded and contracted undergo an atrophy of disuse. Exercise of these muscles will gradually cause them to hypertrophy and regain their former size. Apparently muscle is very dependent on functional use, probably more so than any other tissue in the body.

Regeneration

It is usually stated that the regenerative capacity of mammalian skeletal muscle is negligible. Clark (11) notes that regeneration can occur to the extent of repairing a very limited destruction of muscular tissue. This process of regeneration involves: first, the removal of the necrotic muscle tissue by the action of macrophages; and secondly, its replacement by the development of new fibers—the latter always to be derived as outgrowths which extend from the stumps of the old muscle fibers at the margin of the necrotic area. An important factor in the regeneration of necrosed muscular tissue is

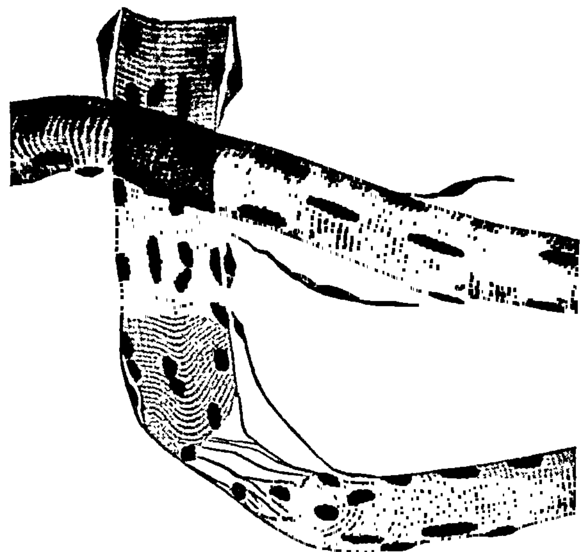


FIG. 137. Two striated muscle fibers of man, in a teased preparation, with stained nuclei. The upper fiber is crushed in its middle and here the sarcolemma is seen. Between the fibers are several spindle-shaped connective-tissue cells. 250×. From A Textbook of Histology, 6th ed., Alexander A. Maximow and William Bloom. Philadelphia & London: W. B. Saunders Co., 1949.

the preservation of the endomysial tubes of connective tissue which enclose the original fibers, because these tubes provide the pathways which guide the newly-growing fibers. In severe muscle destruction a fibrous tissue replacement in the area of injury obliterates the endomysial framework and blocks growth from the stumps of the old muscle fibers at the margins.

Thus, the regeneration in muscle is similar to that in nerve, where the pathways for growing axons may be, and usually are, blocked by proliferative growth of the endoneurum.

LYMPHATICS, BLOOD VESSELS AND NERVES

Lymphatics

Interesting is the silence about the lymphatics of striated muscle, for their absence would be as important as their presence (12) in an effort to understand the situation.

According to Cowdry, Drinker and Yoffey (13) (1941) cite a single author who claims to have demonstrated the presence of the lymphatics in abundance; they go on to say, however, that statements in the literature indicate that these vessels run in fascial planes in and about muscles and further, that the muscular contractions squeeze interstitial fluid out into the vessels for removal. The well-developed lymphatic drainage of cardiac muscle is in sharp contrast, and suggests a difference in the fluid environment of these two types of muscle.

Maximow and Bloom (14) assert that lymphatics have been found in certain muscles within the layers of the perimysium and around the blood vessels but quote no authority for the statement. Le Gros Clark does not commit himself on the subject of lymphatics in skeletal muscle, and Ham (5) states that the lymphatics of striated muscle are confined almost entirely to its thicker connective-tissue components. In this way it differs from cardiac muscle, which has lymphatics as well as capillaries in its endomysium.

The presence or absence of a definite lymphatic system about individual skeletal muscle cells and even within individual muscles is therefore somewhat speculative at this time. Lymphatic vessels are probably present between muscles and muscle groups, and these are perhaps important avenues of drainage and nourishment for skin grafts placed directly over the lower leg muscles after removal of the deep fascia, which serves as a barrier between deep and superficial drainage channels. The author has employed this principle in lymphedema of the lower leg, where the deep fascia over the calf may be completely removed and the edematous and fibrous subcutaneous tissue and overlying skin brought into direct contact with the muscle.[3] Perhaps the improved drainage and nourishment occurring after this procedure is owing mostly to improved venous and arterial supply rather than to lymphatic supply. This same principle has been applied by the author in lymphedema of the arm following radical breast amputation when the lymphatics in the axilla have been interrupted at the original operation. The reduction in edema occurring in these patients is probably due largely to improvement in venous drainage through connections established with the veins in the muscle. When the axillary lymphatics have been removed in the radical breast procedure, the venules in the arm muscle may develop increased capacities to drain off interstitial fluid.

Blood Vessels

Striated muscle, because of its great activity, has need for a rich blood supply to provide an efficient exchange from vessel to muscle cell and vice versa. Arteries are

[3] This is a modified Kondoleon procedure in which all of the deep fascia is removed to prevent regeneration, and no skin or subcutaneous tissue is sacrificed.

carried from the epimysium by the perimysium to the delicate connective-tissue strands of endomysium which surround individual muscle cells. Here in the form of capillaries the vessels are in close relationship with the cells. According to Ham (15), most of the exterior of each fiber is not in direct contact with capillaries. It has been assumed that a thin film of tissue fluid exists between the sarcolemma (cell membrane) of the muscle fiber and the connective-tissue endomysium that carries the capillaries, and that this permits an interchange of dissolved substances between all points on the periphery of the fiber and the capillaries of the endomysium.

The endomysium resembles the delicate connective tissue surrounding individual fat cells, and consists of occasional fibroblasts and their collagenous fibers, which support the capillaries. Occasional elastic fibers are said to be present, as in fascia and tendon.

Blood from the capillaries is collected by venules and then by veins which accompany the arteries. The arteries and veins usually enter the muscle with the branches of the motor nerve.

As stated by Clark (16), injected specimens of muscle show that the branches of intramuscular vessels are freely interconnected by arterial anastomoses, and it might be supposed therefore that a collateral circulation would be readily established following the interruption of one of the channels of blood supply. However, an experimental study of the vascularity of muscle has demonstrated that the anastomoses are not very efficient, so that *a part of a muscle can be functionally devascularized for several days by the ligation of one of its arteries of supply* (17).

This observation is of practical importance since it is probable that *striated muscular tissue is unable to survive if it is deprived of all blood supply for more than a few hours.*

Nerves

The striated skeletal muscles, which are primarily concerned with movements and posture of the body and limbs, receive their motor impulses from the central nervous system by way of craniospinal nerves. The current belief that these muscle fibers receive an additional innervation from the autonomic system is not supported by the most

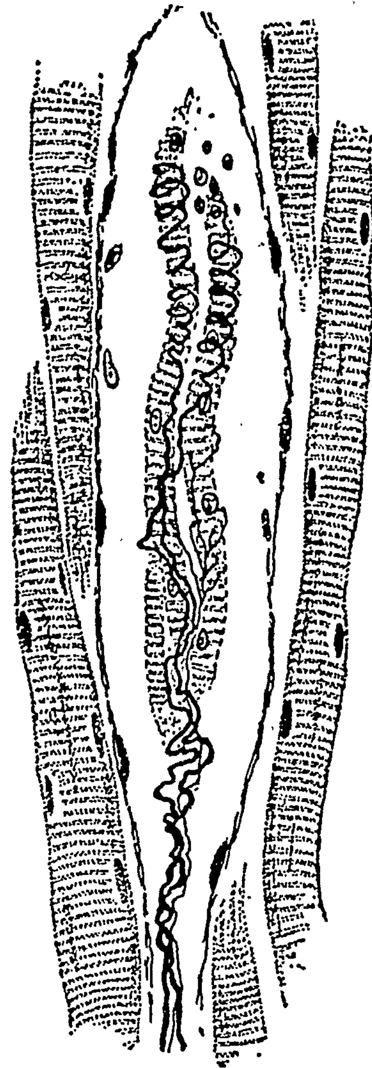


FIG. 138. Drawing of a muscle spindle from the anterior tibial muscle of a mouse. Note the diminutive muscle fibers within the spindle (intrafusal fibers) and the nerve fibers which wind round them in characteristic spirals. The whole spindle is encased in a sheath of connective tissue, and beneath the latter is a 'lymph space'. Magnification ×350 (approx.). From The Tissues of the Body, 2nd ed., Le Gros Clark. Oxford: Clarendon Press, 1945.

careful recent work (18). Each skeletal muscle has a motor nerve whose point of entrance tends to correspond with the geometric center of the muscle.

Muscles which are derived from several rectangular blocks or myotomes in the embryo have motor nerves composed of fibers from several spinal segments.

The branches of the motor nerve form a rich plexus, and terminal medullated nerve fibers innervate *individual muscle fibers*. The nerve loses its myelin sheath on reaching its corresponding muscle fiber, and the delicate neurilemmal sheath becomes continuous with the sarcolemma (19). The naked axon then pierces the sarcolemma and breaks up immmediately into terminal branches which ramify in a local accumulation of granular cytoplasm (sarcoplasm) *containing several nuclei*. This structure is called the *motor end-plate*.

The nuclei of the motor end-plate are derived from those of the muscle fibers; and, if the motor nerve is cut and allowed to undergo complete degeneration, they remain unaffected at first. Cowdry (12) emphasizes that skeletal muscle cells are very responsive to alterations in their living conditions and must have both incentive to work and materials. When the nerve supply is interrupted, skeletal muscle cells very promptly decrease in size. Recovery quickly follows reinnervation if this is not delayed too long but is retarded by immobilization of the muscles.

The axons of single nerve fibers branch on their way to the muscle so that each original nerve fiber innervates a number of muscle fibers. The axon and its delicate branches and their single motor nerve cell, together with the muscle fibers which they supply, are termed a *motor unit*. In limb muscles, which have powerful action, the number of muscle fibers innervated by a single motor nerve cell may be as many as 150 (20). In finely-ground muscles, in which contracture requires to be delicately adjusted for movements of accuracy and precision (such as the eye muscles), the unit is much smaller.

In addition to motor nerve fibers (efferent), muscles also have afferent nerve fibers, which conduct impulses away from the muscle cells. In contrast to motor fibers, which end inside the muscle fiber, the sensory or afferent endings are on the surface of the muscle cell and in contact with its circumference.

Muscle spindles consist of several small muscle fibers enclosed in a connective-tissue sheath and supplied by sensory nerve fibers which terminate by wrapping around the diminutive muscle cells. Muscle spindles are concerned with sensory impulses required to regulate postural tone in muscles and are sometimes called *stretch receptors*.

The possibility of a double motor innervation of striated skeletal muscle by sympathetic as well as somatic nerve fibers led to controversy some years ago.[4] It is generally agreed at present that the histological evidence in support of this conception was misinterpreted. There is evidence, however, that sympathetic activity has an influence on the contracture of skeletal muscle. Clark (21) notes that this is demonstrated by the fact that stimulation of the sympathetic nerve

[4] Le Gros Clark's chapter on skeletal muscle is the best or among the best written on the subject. He combines functional gross anatomy with functional microscopic anatomy in a concise and understandable manner. The reader with more than casual interest should certainly have this book for reference. Cowdry's subject matter is excellent for cytology and interesting historical facts. Maximow and Bloom's discussion is most orderly and scientific, and Ham's, while primarily written for medical students, contains information not found in other texts. The new *Histoloy* edited by Greep is also highly recommended.

Ham is possibly the first academic histologist to recognize that the behavior of tissue cells in free transplants is or should be a part of histology. One might use the term clinical histology for this subdivision of histology.

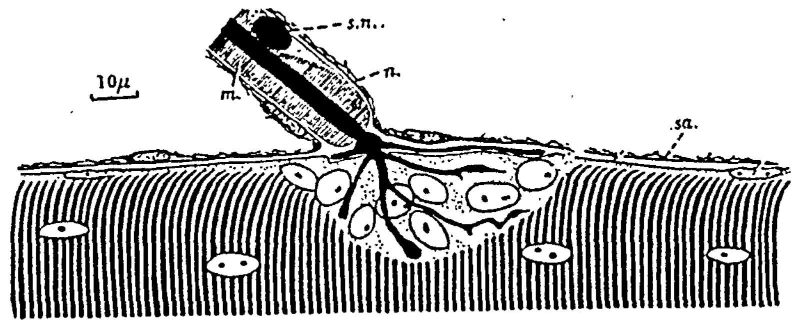


FIG. 139. Diagrammatic representation of a motor end plate. *m.* myelin sheath; *n.* neurilemma; *s.n.* nucleus of Schwann cell; *sa.* sarcolemma. (From E. Gutmann and J. Z. Young, J. Anat. 78: 1944.) From The Tissues of the Body, Le Gros Clark. 2nd ed. Oxford: Clarendon Press, 1945.

delays fatigue in a muscle which is forced to contract by repeated stimulation of its somatic motor nerve. Stimulation of the sympathetic nerve also accelerates recovery in a tetanized muscle. Moreover, this effect is independent of local changes in the circulation.

Clark believes that it is not necessary to postulate a direct sympathetic innervation of skeletal muscle fibers to explain these phenomena, for they may be due indirectly to the liberation of adrenalin (or an adrenalin-like substance) at the endings of sympathetic fibers in the walls of the blood vessels of a muscle (22).

SUMMARY COMMENT ON MUSCLE

The tissue muscle originates from mesodermal cells, which in the beginning appear exactly like the mesodermal cells that give rise to fascia, tendon, bone, and cartilage. (The iris muscle is derived from ectoderm.) Through the activity of some unknown organizer certain of the mesodermal cells become segmented to form a series of rectangular blocks, arranged in a row on each side of the neutral tube. The myotomes or central parts of the rectangular blocks develop into muscle and migrate into position, usually carrying their segmental nerve supply with them.

In its final form skeletal muscle represents the motor for the body, and it differs from other tissues in that its activity can be seen without the aid of magnification.

Skeletal muscle also differs from other ordinary tissue in being multinucleated, perhaps because the length of the cell and its great activity require more than a single nucleus for normal function. In this connection, however, one recalls that a peripheral nerve cell is much longer than a muscle cell, but the former does quite nicely with a single

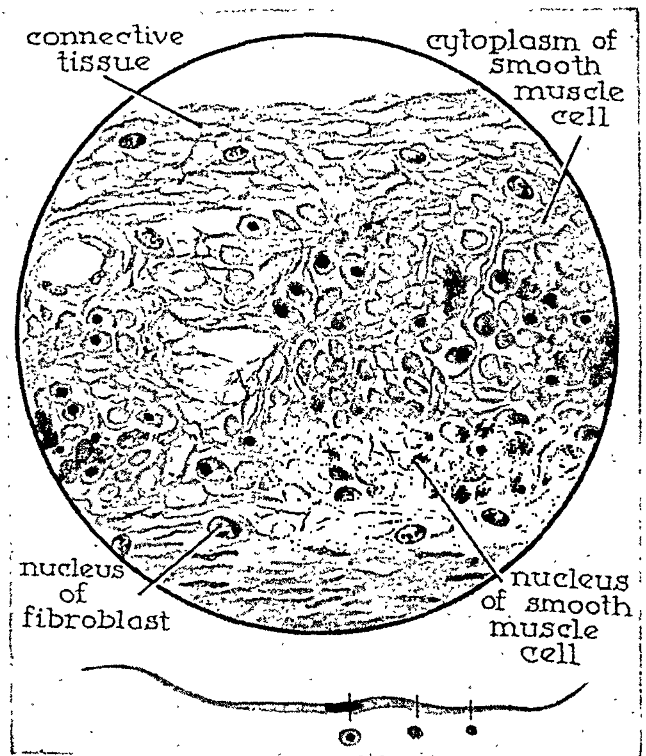


FIG. 140. Drawing of a group of smooth-muscle (high-power) cells cut in cross section. Since smooth muscle fibers are elongated, cross sections cut through them seldom pass through their nuclei. Hence most of the smooth muscle fibers seen in this illustration do not reveal nuclei. Those that are present are either centrally or eccentrically disposed. The single fiber illustrated in the lower part of the picture shows how cross sections through different parts of a fiber appear. From Histology, Arthur Worth Ham. Philadelphia: J. B. Lippincott Co., 1950.

nucleus despite great activity and a high metabolic rate.

Skeletal muscle is extremely sensitive to physiological changes and it does not thrive when its normal surroundings are altered. It must, in actual fact, work to live, and it must constantly have necessary materials for work and adequate elimination of waste products or it will degenerate rather quickly.

The skeletal muscle cells receive motor and other impulses through efferent fibers and send out afferent impulses which stimulate a motor or other response by means of the well-known reflex arc.

The general pattern of nerve waves reaching skeletal muscle through the motor or efferent nerve probably have many functions other than that of simple contracture. Posture and tone are known factors but undoubtedly there are others.

Now it is well known that a skeletal muscle will atrophy *in situ* when its motor nerve is cut. In the past, crude electrical stimuli have been used to make the paralyzed muscle contract, but despite this stimulation the muscle cells eventually degenerate and fail to respond unless axons from the motor nerve reestablish continuity with nuclei in the motor end-plate. Perhaps continuity of the afferent sensory nerves is also important. At any rate it would be interesting to duplicate the various wave lengths found in the pattern normally reaching the muscle, and send these to the muscle directly or through the distal end of the cut motor nerve. Perhaps atrophy of the muscle cells would be delayed, or, happily, might not occur if this could be accomplished.

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CHAPTER 28

Transplantation of Muscle in Animals

The first step toward attempts in muscle transplantation was study of the regenerative process following muscle injury. Later investigators became interested in the phenomenon of muscle atrophy following severance of the motor nerve supply and attempts were made to substitute artificial electrical stimulation for the muscle to prevent atrophy. The rapid degeneration and replacement of muscle after its blood supply had been interrupted were also a problem of great interest both to the clinicians and to animal experimenters.

There is a tendency in the literature, especially in animal experimentation, to present rather ponderous research work which is the opposite of succinctness and clarity. A great multitude of factual evidence and theory is presented in such a way that the reader is confused and is apt to lose interest in the subject.

In this review of the contributions on muscle transplantation the author has tried to accurately present the findings and interpretations as written by various investigators. Numerous foreign articles were reread several times in order to avoid errors in translation from one language to another, and many were not included because of their obscurity.

The reviews presented represent a random sampling of the literature on the subject of free muscle grafts and muscles transplanted with intact nerve and blood supply.

It seems that Waldeyer (1), of the University of Breslau, in 1865 was among the first who attempted to investigate the regeneration of cross-striated muscles in a defect, using frog muscle for his experiments. He accepted the development of primitive bundles from several cellular elements. In the fifth week after injury, beside the granular degeneration there was an area of glossy muscle fibers. Waldeyer noted nuclear proliferation in all stages of change, degeneration of sarcolemma, and the formation of round and spindle-shaped cells in the inner perimysium.

Weber (2) of Heidelberg in 1867 pointed out that in old text books it had been repeatedly maintained that muscle wounds heal only through connective tissue. He made a cross-cut in, and removed a piece out of, the M. tibialis anticus in rabbits and examined preparations at intervals of 4 to 120 days after injury. Other experiments on dogs and cats and some observations on humans verified his findings on the process of new muscle formation. He noted changes in the old muscle fibers and in their vicinity and the development of young muscle fibers and sarcolemma, in conical form, from the cut ends of the muscle bundles. The muscle nuclei increased by division. Band-like ele-

nucleus despite great activity and a high metabolic rate.

Skeletal muscle is extremely sensitive to physiological changes and it does not thrive when its normal surroundings are altered. It must, in actual fact, work to live, and it must constantly have necessary materials for work and adequate elimination of waste products or it will degenerate rather quickly.

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destroyed. There was no evidence of viability and no indication of progressive development. Sections were made in the M. gastrocnemius in hens and homografts of similar muscle with tendon were implanted. The implanted muscle was completely absorbed. *Thus, Magnus, after careful histologic studies, refuted the assumptions of Gluck and Salvia.*

Muscle was transplanted into the brain of animals by Askanazy (9) (1891, 1912) and regeneration was noted at the end of the first week postoperatively. At the beginning of the third week signs of atrophy were observed in most of the newly-formed fibers. Only a few fragments of the transplanted muscle fibers showed a fibrillar structure after two months.

Volkmann (10), of the Pathologic Institute of the University of Marburg, in 1893 replaced excised pieces of thigh muscle in the same site or on opposite legs of rabbits and dogs, and made extensive observations from 3 to 60 days. He also studied muscle regeneration in human muscle. In his classic study he noted that regeneration of striated muscle tissue always comes from the nuclei of old fibers. In direct relation with the old fibers the regeneration in general is similar to the embryonal type of muscle fiber formation. When without direct connection with the old fibers, the formation of young elements is by the budding process. In both cases proliferation of the muscle nuclei and of the protoplasm surrounding these nuclei represent the beginning of the regenerative process. He believed that free muscle transplants never remain viable but die immediately and are later absorbed. In their place scar tissue occurs, which shows partial muscularization like every other muscle scar. *Volkmann's conclusions therefore agreed with those of Magnus and opposed the earlier conclusion made by Gluck and Salvia.*

At the International Congress at Moscow in August 1897, Rydygier (11) maintained, on the basis of experiments on two dogs, that successful transplantation of pedicled muscle flaps was possible and gave a demonstration of microscopical preparations.

In a series of experiments on dogs, Capurro (12) in 1900 reported favorable results from transference of pedicled muscle flaps. In a second series both rectus muscles were exposed and a free muscle graft was placed between the muscle ends on the opposite side. He concluded that free implantation of cross-striated muscle grafts, in animals of the same species or in animals of a different species, gave negative results. The disintegration of the tissue took place rapidly in the majority of instances through the process of ischemic necrosis; in some there was caseation of the free muscle graft; in others, fibrous metamorphosis. The transplantation of pedicled flaps is for the purpose of mechanical support or for strengthening the part and maintaining functional ability of special tissue.

In an extensive series of free autoplantations of muscle in rats and mice, Saltykow (13) (1900) observed that at first the transplant degenerated. After five days there was evidence of far-advanced regeneration, the muscle nuclei having increased in number and in size. The contractile substance around the nuclei showed degeneration in small granular masses and disappeared. Spindles gradually formed, from which came narrow muscle fibers. The fibers near the periphery were either best preserved or showed the greatest regeneration because of their nearness to the blood vessels of the adjacent tissues. In two weeks following transplantation the fibers had grown in width and the nuclei were less numerous in the periphery of the fibers. The presence of cross-striations was suggested in a few fibers. After four weeks the new fibers showed a difference from the original ones only by a slight narrowness. A simple atrophy occurred during the later stages. Scarcely any cross-striated muscle fibers could be seen at

ments were only the intermediate stages between the outgrowing muscle spindles and the spindle-formed muscle cells with granular protoplasm or beginning cross-striation. Weber held the formation of the sarcolemma to be a product of the connective-tissue cells which grew between the young bundles in a parallel arrangement.

Neumann (3) of Königsberg in 1868 made a study of the gastrocnemius and tibialis anticus after simple incision, and resection of muscle pieces in a series of experiments on rabbits. Examination of the injured muscle was made at different periods varying from 1 to 175 days postoperatively. He found that the healing process essentially arises in a growing-in of the sectioned muscle fibers into the scar tissue until the ends become attached to each other. It was his belief that in the course of healing, with or without substance loss, new formation of muscle substance occurs.

Neumann incised the tibialis anticus muscle in a rabbit and examined the scar 6½ weeks after the nervus peroneus had been sectioned. The muscle was thinned to a high degree, was of pale yellow color and severely degenerated. Microscopically the interstitial connective tissue of the muscle was well developed and was penetrated by fat cells. The fibers were small, their contractile substance granular, without definite cross-striation; the margins were uneven, and the sarcolemma was without sharp line, the nuclei being richly proliferated.

Kraske (4) in 1878 considered the muscle cell tubes as derived from proliferated sarcolemma nuclei. He then showed that the cells increased longitudinally by direct nuclear division and at the end of the third week became transversely striated.

REVIEW OF LITERATURE ON ANIMAL MUSCLE GRAFTS

Zielonko (5) is said to have produced the first work on the free transplantation of striated muscle in 1874. He transplante muscle tissue into the lymph sac of a fro and observed rapid necrosis of the graft an no evidence of regeneration.

Gluck (6), of the Surgical Clinic at th University of Berlin, in 1881 reported su cessful implantation of pieces of free muscl with and without tendon, from the rabb and hen (heterografts and homografts) int defects in hen musculature. Forty days afte operation he observed healing in place, an energetic contractions when the implante muscle as well as the nerve were stimulate through faradization. He noted a regenera tive process in the implanted muscle whicl following active infiltration, was converte into a fibrous mass in a few instances. N necrosis was seen in a large number of myo and tendoplasties. Gluck concluded that fre transplantation of muscle succeeds unde precaution to avoid crushing and with car in suturing.

Salvia (7) in 1883 reported successfu transplantation of homogenous muscle graft in dogs and rabbits. He found no difference between the transplant and the surroundin muscle, either grossly or microscopically after a period of 3 months postoperatively He believed that the transplanted muscl fibers lost their anatomical properties in tha they assumed those of the animal in whon they were transplanted.

Magnus (8) (1890) made small incisions i the M. quadriceps femoris or tibialis anticu in rabbits and also implanted into defect produced in the quadriceps muscle pieces o similar muscle from the same or anothe animal. His observations extended over a period of 7 to 60 days postoperatively. *The implanted muscle as an autograft and homograft degenerated.* Microscopically, the specimens showed exfoliation of muscle fibrils disintegration, and granular detritus. Round cells from the surrounding muscle part penetrated into the muscle implant. The connective tissue and blood vessels were also

generation took place from the host site, long small muscle fibers extending into the connective tissue of the transplanted muscle piece. Regeneration also went on from the transplanted muscle tissue, by means of giant cells, plates rich in nuclei, muscle plates, sarcoblasts and so on, in the margins. Jores seldom saw terminal bud formation. From the disintegrated transplanted tissue came a regenerative new formation. In the late stages nothing more of the original transplanted tissue was present. He concluded that faradic stimulation of these muscle pieces increased the regeneration of the peripheral muscle fibers and the preservation of parts of the transplants temporarily.

Schmid (20) (1909) attempted to ascertain the effect of functional stimulation on free muscle grafts 6 to 8 hours after transplantation. A weak faradic current was applied as frequently as 6 to 7 times daily. Schmid held that the stimulated muscle transplant healed in place and, despite degeneration being observed, regeneration of the muscle fibers took place. The graft and the normal muscle of the host did not differ grossly after 2 months. It was further noted that the muscle did not regenerate in grafts that had not been electrically stimulated.

Askanazy (21) (1912) concluded that for different organs the transverse muscle substance is successfully transplantable. However, a large part of the transplant becomes necrotic, and regeneration takes place from the retained living muscle cells on the periphery of the graft.

Landois, and Haberland (1913) studied the processes in free transplanted muscle under careful microscopic control. Landois (22) found that the free muscle transplant leads to necrosis and is therefore dangerous as well as purposeless. There was no success even with faradic stimulation. In favorable cases there was formation of young muscle elements in the fibers of free muscle transplants.

Haberland (23) established that free transplanted muscle tissue heals in but that most of the muscle fibers are destroyed since nourishment is not provided. At the periphery, regeneration and nourishment of the fibers by osmotic processes are always observed.

In a study of the behavior of muscle transplants in dogs, Shinya (24) (1914) histologically compared autografts and homografts of cross-striated muscle transplanted through a longitudinal split in the perineurium of the sciatic nerve substance, the perineurium being sewed up with human hair to prevent the extrusion of nerve substance and the transplant. His conclusion was that muscle can be transplanted in the same individual or in others of the same species with good results. The lymphocytic reaction was observed to be much stronger in the homotransplant than in the autotransplant, and muscle fibers were maintained much longer in the autotransplant. In addition, new formation of muscle fibers was much less intense and degeneration in the newly-formed fibers was much earlier in the homotransplant than in the autotransplant. In 35 days postoperatively the autogenous muscle had disappeared entirely and had been replaced by connective tissue. In 27 days necrotic dark red-stained pieces of homotransplant were visible, in which many leukocytes had accumulated. Shinya concluded that the manner of formation of new muscle fibers showed a difference in the autotransplants and homotransplants. In the autografts single sarcoblasts were observed in the main along with cellular chains of syncytial elements, whereas the multinuclear buds of muscle predominated in homografts.

In experiments on rabbits Heineke (25) (1914) showed that motor impulses could be directly transmitted to muscle when the

the beginning of the fourth month. Saltykow believed lack of function was the cause of the final atrophy.

After experimental investigation Kroh (14) in 1905 stated that faradic stimulations could not prevent the degeneration of free muscle transplants. In 6 to 8 weeks after transplantation the degenerated muscle tissue is replaced. A granulation tissue of fine fibers, poor in vessels, as well as regenerated muscle fibers developed, originating not only from the peripheral zone but also partly from the transplant itself. Kroh believed that the regenerated muscle tissue was destroyed later due to lack of nerve impulses. A connective tissue rich in fat and inclined to shrinking is the eventual fate of every free muscle transplantation.

It was Hildebrandt (1906) who recognized the importance of the preservation of the nerve supply in muscle transplantation. In his experimental muscle transplants, however, in which the nerve was left attached, viability was not maintained. Microscopically, degeneration began within a few hours. The fibers became swollen, the striation disappeared, and the nuclei showed degenerative changes. Infiltration of round cells and granulation tissue were seen. At the edge of the transplant a few of the fibers remained well preserved for some time. Finally the transplant was absorbed and replaced by connective tissue (15).

Schminke (16) in 1908 made an exhaustive investigation of the regeneration of transverse muscle in ichthyopsida and sauropsida. He confirmed new formation tending to the more or less complete replacement of a wound defect, especially in lizards and birds. In fish, tailless amphibia, reptiles, and birds new formation proceeded from elements of old fibers, through terminal budding of the fiber, or the fiber was divided longitudinally into fibrils which became swollen at the ends into buds.

The experiments by Caminiti (17) (1908) at the Surgical Clinic of Naples were concerned with muscle transplants of the extremities and tail of dogs, especially the biceps brachii. For success in procedure the nerves and vessels of the muscle, the muscle sheath and aponeurosis must be avoided. The free pieces of muscle should be fresh and quickly transplanted, with contact over large surfaces. Eleven out of 20 animals showed positive functional results. The traumatic loss of muscle was compensated for by connective tissue, as were the muscular wounds.

Dawson (18), of the Pathologic Department of Edinburgh University, in 1909 reported on his findings when wounds were made in the rabbit and white rat through the whole thickness of the anterior abdominal wall. The animals were examined after periods varying from a few hours to the time of complete healing of the wound. Dawson concluded that the degenerative and regenerative processes were very closely associated. Early proliferation of nuclei occurs in all parts of the preserved muscle fiber within the area of reaction. "Muscle cell tubes" are formed from the second day onward. Regeneration may take place to a slight extent from the ends of preserved muscle fibers by "budding" or cleaving off of spindle-shaped nucleated elements.

Jores (19) (1909) transplanted a piece of thigh muscle into the dorsal musculature under slight tension in the same rabbit. In the main the transplanted muscle piece soon became necrotic and was extruded. When free muscle autografts were stimulated by electric current 6 to 7 times daily, contraction occurred. Grossly, muscle pieces healed in. Microscopically, a few larger muscles did not change in appearance; others were necrotic and appeared wider and homogeneous. There was no nuclear staining. Other muscle bundles were penetrated with connective tissue rich in nuclei. Still others were completely converted into connective tissue. Re-

necrosis of all the tissue at the center of the injection, and in hyaline degeneration of the contractile substance of the muscle fibers at the periphery of the injected zones. In a subsequent repair process necrotic material was removed by phagocytic cells, and the destroyed muscle was replaced by scar tissue containing some regenerated muscle fibers. Forbus considered the phagocytic cells found within the persistent sarcolemma as wandering cells of extramuscular origin and having no connection with the muscle cells. Regeneration of the muscle is brought about by cells which develop from the nuclei and sarcoplasm of the old preserved muscle fiber. The new fiber, Forbus held, appears to arise either from a single muscle cell or from fusion of several such cells. Vital staining was insufficient for differentiating between cells of muscular origin and cells of extramuscular origin.

In experiments on rats reported by Elson (30) (1929) a piece of the xyphoid cartilage with a part of the cross-striated muscle attached to it was transplanted either into a subcutaneous abdominal pocket of the same rat or of another rat of a different breed. Each animal received both an autotransplant and a homotransplant in two different subcutaneous pockets. Pieces were removed and examined histologically after varying periods ranging from 2 to 118 days.

Striated muscle in autografts can survive in the host at least for a period of 4 months without functioning. That is to say, well-preserved muscle was found as late as 118 days after transplantation. There was less regeneration of the muscle tissue in homografts in the first period after transplantation and much more degeneration owing to invasion by lymphocytes and, to a less degree, by connective tissue at subsequent periods. Homotransplanted muscle disappeared much more quickly than autotransplanted muscle. It is thought that the homotoxins may injure the muscle tissue directly, diminishing the regenerative process and increasing the subsequent degeneration.

There is no true outgrowth of muscle fibers anywhere. The major portion of the old muscle tissue undergoes regenerative processes following primary degeneration. The sarcoplasm increases and a proliferation of its nuclei occurs. Subsequently the sarcoplasm differentiates into typical striated muscle, in which cross-striations are again formed. The nuclear proliferation becomes progressively less active; the nuclei decrease in number and are seen in the periphery of the fibers, and the fibers increase in size. Ultimately almost normal muscle fibers are again present. Elson did not regard the growth process as regeneration in the strict sense.

In an artificially-produced fistula in dogs Pool and Garlock (31) (1929) fashioned a pedunculated flap of the latissimus dorsi muscle, corresponding to the size of the fistula, in the form of a tube and then placed it deeply into the bronchus. The remainder of the muscle flap was anchored to surrounding structures. In two dogs the results after 10 months and a year respectively indicated processes of repair. The muscle flap remained viable and was not completely replaced by fibrous tissue. Microscopic examination showed intact muscle fibers at the end of a year. There was growth of bronchial epithelium over the muscle flap. Clinically this method used in several patients with persistent bronchial fistula met with success in their hands.

In experiments conducted by Bartoli (32) (1930) a part of, or an entire, lateral gemellus of one rabbit was implanted into the gemellus muscle of another, with a pedicled graft, leaving the tibial nerve intact. When the rabbits were sacrificed from 2 to 4 days after the operation direct examination showed that the pedicled graft was adherent to the host muscle, where it appeared normal in

peripheral motor nerve was implanted into the paralyzed muscle tissue. Thus he was able to obtain contraction in rabbit muscle by faradic stimulation of the implanted nerve as early as 8 to 14 days postoperatively. That an outgrowing motor nerve can penetrate into muscle without an old nerve pathway, and can form end-plates was demonstrated by Heineke in direct transplantation of a nerve in the muscle.

In a series of experiments on guinea pigs carried out by Erlacher (26) (1915), a flap of the biceps brachii was separated in such a manner that the nerves were sectioned and the lumina of the blood vessels were lying adjacent to one another. The muscle underwent rapid degenerative changes, and all the nerves in the separated muscle part degenerated shortly and became absorbed. A separated muscle flap could be richly provided with nerve elements from the healthy muscle lying beneath it. The outgrowing young fibers could easily find the adjacent lumina of the old sheaths. The fibers which had grown in an old pathway advanced most rapidly, but a large number of young nerves spread directly on adjacent muscle fibers, creeping along, then penetrating and forming an end-plate. For this penetration of a motor nerve into a muscle fiber the old nerve sheath was not absolutely necessary. In 6 weeks the muscle tissue could be regarded as functionally and anatomically reestablished.

In another series a free muscle of the right biceps was transplanted to the left biceps, and vice versa. In 25 days the free transplanted muscle healed in without reaction. Microscopically, the transplants showed a healthy part with normal appearance, a very large number of young newly-formed nerve fibers penetrating toward the transplant or into it. The separated nerve tissue always completely disintegrated. Nourishment from without protected the transplant until new nutritive connection formed. Through rapid occurrence of new connections *the muscle tissue itself, from stages of extreme degeneration, can regenerate to muscle tissue able to function, and it is not replaced by connective tissue.* Twice as much time is necessary for this process, because regeneration of the muscle takes place only after regeneration of the nerve. Erlacher called attention to the fact that he used only small pieces of muscle in his experiments. Implants into normal muscles always gave isolated contractions when faradized.

Steindler (27) (1915) divided the anterior crural nerve and isolated the sciatic nerve, one longitudinal split half of the sciatic nerve being inserted into the paralized M. rectus femoris in dogs. Sixteen weeks postoperatively the muscle showed complete regeneration in all parts.

In dogs and cats Steindler (28) (1916) found direct neurotization to be possible. The natural limits of physiological regeneration allow a motor nerve, directly implanted into paralyzed muscle tissue, to establish the entire chain of neuromotor connections by regeneration. This regeneration becomes complete in 8 to 10 weeks after implantation. Muscle tissue also regenerates; the fibers having a normal contour and normal striations.

In experimental work by Forbus (29) (1926), rabbits and dogs were given injections of dye intravenously, and white rats and guinea pigs intraperitoneally, to stain all the superficial tissues. Injury was produced by cutting off the blood supply to a whole muscle in rabbits and dogs and the muscle left in anemic state for 3 hours. The ligatures were then removed and the wound closed. Muscle lesions were also produced in rabbits and pigs by injection of irritants. After a maximum period of 14 days the injured muscles were excised at intervals and sectioned. In other experiments variations of this procedure with and without staining were carried out and observations made.

Damage to muscles by irritants resulted in

necrosis of all the tissue at the center of the injection, and in hyaline degeneration of the contractile substance of the muscle fibers at the periphery of the injected zones. In a subsequent repair process necrotic material was removed by phagocytic cells, and the destroyed muscle was replaced by scar tissue containing some regenerated muscle fibers. Forbus considered the phagocytic cells found within the persistent sarcolemma as wandering cells of extramuscular origin and having no connection with the muscle cells. Regeneration of the muscle is brought about by cells which develop from the nuclei and sarcoplasm of the old preserved muscle fiber. The new fiber, Forbus held, appears to arise either from a single muscle cell or from fusion of several such cells. Vital staining was insufficient for differentiating between cells of muscular origin and cells of extramuscular origin.

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color. In rabbits sacrificed 20 to 30 days after operation, although the connections between the graft and the host tissue were quite well linked, the color became abnormally yellow. Forty days after operation the whole muscle transplant appeared yellow, and as time progressed the graft started to lose its vitality.

In one group of rabbit experiments by Comolli (33) (1931) the tissue was taken from a part of, or from an entire, lateral gemellus and implanted into the other lateral gemellus muscle. The central head of a sectioned peroneus nerve was implanted into the graft or sutured to its nerve. The tibial nerve and the nerve of the host lateral gemellus muscle remained intact. In another group of rabbits the conditions were the same as in the first group except that the tibial nerve and the nerve of the host lateral gemellus muscle were cut. The graft took in both the partial and entire muscle implantations. The tissues of the graft and those of the host assume intimate reciprocal connection, in part by regeneration of muscular fibers from one another, and in part by formation of a connective scar between the graft and the host tissue.

In pedicled autoplastic grafts made by Dainelli (34) (1932) in rabbits, the tissue was taken from part of a muscle close to the host muscle and implanted into the anterior lateral surface of the quadriceps crureus. The dissection was made downwards directly parallel to the direction of the muscle fibers. The grafted tissue was examined microscopically after 6 to 110 days. Free homoplastic grafts taken from the anterior lateral surface of the quadriceps crureus of one rabbit was transferred to the corresponding muscle in another rabbit, with and without injections of the donor's muscle extract before and after transplantation. Sections were examined 8 to 60 days postoperatively. Macroscopic and histologic observations showed that the homoplastic muscular transplants did not succeed, either when done under normal conditions or with the use of injections; they became completely replaced by connective tissue. The autoplastic pedicled transplants underwent complete fibrous transformation, so that the results were completely endangered. Dainelli explained the failure of the homografts to take as due to histologic conditions of the host and graft tissues, humoral factors, and conditions of innervation and vascularization. He attributed failure of autotransplants to the above factors and in addition to muscular hypotrophy and loss of the vitality of the graft, to the penetration of connective tissue between the muscular elements, and to the progressive replacement of the graft by connective tissue.

At a society meeting Leriche and Fontaine (35) (1933) presented the heart of a dog in which they had taken off a large strip of pectoral muscle (10 by 6 cm.) to serve as a graft and applied it for infarct of the myocardium, and aneurysm. The animal was in excellent condition when sacrificed. The pericardiac cicatrix was supple. At the site of the graft the scar adhered closely to the ventricular wall. When the heart was freed, the muscle graft had the appearance of being completely incorporated and was no longer distinguishable from the neighboring wall except by a slight relief. *In section it was difficult to distinguish the graft from myocardial tissue.* The yellow coloration, however, was different from that of a healthy muscle. There was no deficiency visible in the wall. Leriche and Fontaine thought that the result obtained experimentally would tentatively justify the risk of the operation on the human.

In experiments on rabbits, von Seemen (36) of the University Clinic of Munich (1934) sectioned the nerves supplying the M. rectus abdominis. At histological examination made at varying intervals, increasing atrophy and degeneration of the abdominal

muscles separated from the nerve stumps were observed. The interstitial connective tissue proliferated only slightly and then fat tissue was laid down, replacing the deficient muscle mass. Observations over a period of three years showed that the neurotization in the neighborhood of the cut nerve did not progress further, and the musculature separated from nerve branches largely disappeared and was penetrated by fat tissue cells. This severe injury of muscle function was shown clinically by greater arching of the operated half of the abdomen and through loss of ability to contract in response to electrical stimulation. In subsequent operations preparations made from human muscle parts which had been separated from their nerve branches by operative sectioning showed a high degree of atrophy, associated with cloudy hyaline degeneration.

Millar (37) (1934) exposed the left rectus femoris muscle in young rabbits and crushed through or incised the belly of the muscle, the fibrous sheath being kept undamaged. After varying periods of 1 to 33 days the animals were sacrificed and kept in refrigeration at 4°C. until contractility of the muscle ceased. Millar found that in the young rabbit regeneration of muscle fibers of approximately perfect structure takes place from the damaged ends of otherwise intact muscle fibers and not from individualized cells nor from muscle giant cells. New myofibrils are laid down in the cytoplasm of the muscle bud, probably in continuity with existing myofibrils, which is associated with "a striking and apparently hitherto undescribed change in the nucleolus." The division of the muscle nuclei appears to be amitotic.

Tiegs (38), of the University of Melbourne, in 1934 reported on a large quantity of material of vertebrate origin regarding the structure of striated muscle fiber. In large muscle fibers of vertebrates and of arthropods the striae are the optical expression of a helicoid and not of a succession of transverse discs. The helicoid may be double or single. Various forms of "helicoidal" arrangements occur. Forms of muscle fiber exist, especially in invertebrates, where the helicoidal arrangement does not take place.

In a study of regeneration after impairment in muscle tissue, Schmincke (39) (1936) found that the new formation comes only from the fibrous tissue at the margin of the injured area, through discharge of a sarcoplasmic substance as homogeneous irregular club-shaped processes. Into these the sarcolemma nuclei grow by amitotic increase. The longitudinal and cross-striations develop in continuity with fibrils present in the old fibers and seldom within the buds. In connection with this continuous regenerative process there is a second, discontinuous one, which has a course like the embryonal development. On the basis of a great deal of material examined, Schmincke had the impression that the formation of terminal buds is the most frequent process of new formation of fibers in humans.

In experiments on rabbits, von Gehlen (40) (1937) replaced autogenous pieces of muscle—M. vastus lateralis—in the defect produced by their removal. He found that healing in occurs, after previous degeneration, by means of regeneration essentially in the embryonal manner. *The newly-formed fibers of the transplant are permanently maintained.*

Spinal nerves three, four and five of the brachial plexus in the Japanese newt were severed in the quantitative studies made by Weiss (41) (1937). After a period of 120 days nerve counts were made in cross section at three levels. The indication was that the branching of regenerating fibers is extensive enough to permit reinnervation of a limb even from an undersized nerve source. "The degree to which the limb is repleted with regenerated fiber branches is controlled by

factors residing within the limb and tending to limit the actual supply to an approximately normal amount. The degenerating peripheral nerves may represent one major factor exerting numerical control over admission of new fiber branches."

McNealy and Shapiro (42) (1937) used pedicled muscle grafts from a neighboring portion of the sternomastoid to repair the longitudinal or transverse slits in common carotids, and other muscle flaps to repair both femorals in dogs. In 1 to 8 months postoperatively the animals were sacrificed. In every instance in which the pedicled muscle graft was sutured into the incised artery the graft took. The viable graft was found attached to the muscle at one end and to the wall of the artery at the other end. Closure of arterial wounds by a pedicled muscle patch produces hemostasis and permits healing of the wound without histological impairment of the lumen.

In a preliminary report Livermore (43) of the University of Tennessee (1939) stated that pieces of fat and muscle were transplanted into incisions made in the upper and lower poles of the kidney respectively, and the kidney replaced in the abdominal cavity. His deductions were that foreign material introduced into the kidney parenchyma causes infarction, deposition of calcium, and damage to kidney tissue, the muscle transplants producing more damage than fat. Both muscle and fat undergo degeneration and replacement by connective tissue.

Hines and Knowlton (44) of the University of Iowa (1939) reported on determinations made of concentration of chloride, fat, and water in the gastrocnemius muscles and serum from seven groups of rats. From these data calculations were made on the relative masses of connective tissue and muscle cell phases present in muscles at various ages. Values characteristic of adult tissue are found at 90 days of age. After that approximately a constant composition was noted. The calculated value for the connective-tissue phase shows a range of 40 per cent of the total mass at 15 days to 15 per cent at 90 days of age. The calculated percentage of water in muscle cells decreases during the growth period.

Chèvremont (45) (1939) of the University of Liége utilized the muscles of the foot of chicken embryos for culturing. The muscle buds which appear in the skeletal muscle consist, in general, of several slender strips. Each strip issues from alteration in a muscle fiber of the tissue culture. The buds are fragmented or are transformed into cells of a specific character. These cells can be transformed into macrophages. The latter can also arise directly from the buds.

As investigated by Sperry (46) (1940), the M. tibialis anterior and the M. extensor digitorum longus were cut and inserted as autogenous pedicled grafts onto the Achilles tendon of the heel of albino rats, so that in their new position contraction of the muscles produced plantar flexion instead of dorsiflexion. Every other muscle of the shank except the M. peroneus longus was removed. In some animals the transposition of flexor extensor muscles of the foot produced a reversal of foot movement. There was no functional adjustment. Mechanically the transposed muscles were capable of producing normal movement when the nerve discharges were properly timed. The sensitivity of the shank, ankle, foot, and transposed muscles and tendons was not impaired by the operation. No reeducation was possible in rats kept for 5 to 15½ months postoperatively. Sperry concluded that there was indication of a central nervous organization of the basic motor patterns for limb coordination.

In experiments on wound healing, Chouké and Whitehead (47) (1941) used the pectoralis major, the external oblique, the rectus abdominis and others for suturing muscle to muscle in dogs and rats. Suturing

was at right angles to the incisions, and muscle edges were closely approximated without causing tension. Examination of the muscle in the operated tissue was made at varying periods of 8 to 142 days. After 16 days complete repair seemed to be present. Healing of severed striated muscle occurs, as viewed by Chouké and Whitehead, by fibrous connective-tissue growth from the epi-, peri- and endomysium, and not through regeneration of muscle cells.

Naffziger and Aird (48) (1942) observed progressive degenerative changes in nerve endings, end-organs, and muscle fibers in the latissimus dorsi after ligature of the thoracodorsalis nerve in the axilla and in the muscle of dogs. In resuturing the nerve the cicatrized portion was resected and fresh ends in good condition were anastomosed by two or three arterial silk sutures through the nerve sheath. Serial biopsies, taken after 2 and 3 weeks and monthly, showed a progressive loss of muscle cells and a terminal development of fibrosis. Serial biopsies of the same muscles in a regenerative phase, instituted by anastomosis of the thoracodorsalis nerve, showed no appreciable variation in the amount of regenerating nerve endings or end-organs.

In animal experiments Hines (49) (1942) produced complete paralysis of the muscle by crushing the tibial nerve at the level of its junction with the peroneal nerve, in albino rats. Partial paralysis was produced by section of one or more of the spinal nerves contributing motor fibers to this muscle. Quantitative measurements of the muscle mass, strength, and creatine content were included. In two weeks following nerve crushing the muscle lost weight, strength and creatinine at a rate precisely similar to that following nerve section. Immobilization by casts was found to retard recovery from paralysis. This retardation in recovery appears to be due to a decreased rate of muscle regeneration rather than an effect of nerve regeneration. The overall effects of natural and artificial activity are in the direction of an improvement in rate and extent of recovery from peripheral nerve paralyses. There was no evidence that axon branching could be utilized to enhance the reinnervation of partially paralyzed muscle.

In the study of regeneration by Hines, Thomson and Lazere (50) (1942) a lesion was produced by crushing the tibial nerve of one limb in albino rats at the level of its junction with the peroneal nerve, giving complete axone separation and paralysis of the gastrocnemius muscle. Most of the studies were made at varying periods from 12 to 84 days after denervation. Regeneration was slower in older than in younger animals. The earliest signs of functional reinnervation appeared at 12 to 15 days after denervation. At about this time fibrillary activity had ceased but an increased sensitivity to acetylcholine was noted for as long as 35 days. Muscle strength to direct and indirect nerve stimulation increased rapidly after initial reinnervation until the thirty-fifth day. Subsequent recovery of strength was slower and was considered largely due to increase in muscle mass. "The data for the rate of regeneration of muscle weight were found to fit a formula essentially that expressing the body growth rate." At 84 days the muscles in the process of regeneration were found to possess from 80 to 90 per cent of the creatinine content, weight, and strength of their contralateral controls.

Muirhead and Hill (51) studied shock resulting from intraperitoneal implantation of muscle. An autogenous segment was passed through a grinder, desiccated in the frozen state and stored for several weeks. A thick paste of this ground muscle introduced under pressure into the peritoneal cavity was capable of producing fatal shock in dogs. The changes in the viscera were identical to those after freezing shock, except

a dilution of the circulating plasma proteins was observed. Muirhead and Hill found evidence favoring the absorption of shock producing substances, since after a period of time the muscle substance lost its shock-producing qualities.

Minced homogenous muscle in saline was implanted into the peritoneal cavity of dogs by Fowler (52). Hemoconcentration, a fall in blood pressure, tachycardia, and vomiting were observed a few hours after implantation. Autoclaving of the muscle before implantation overcame complication by *Cl. welchii* infection. A greater amount of autoclaved muscle was required to produce shock. Large quantities of desoxycorticosterone acetate and aqueous extracts of adrenal cortex had no beneficial effect on the shock.

Hines and Wehrmacher (53) (1944) carried out experiments on the gastrocnemius muscles of more than 2000 cats, guinea pigs, and albino rats, in which the muscle of the hind limb was denervated or tenotomized unilaterally or bilaterally. Electrical stimulation delayed denervation atrophy only when conditions permitted the muscles to develop effective tension. Inactivity and immobilization retarded neuromuscular regeneration. Artificial stimulation and exercise exerted a favorable effect on regeneration. Hines and Wehrmacher believe, on the basis of the evidence obtained, that early muscle use is the best means of accelerating recovery from peripheral nerve injury.

According to investigations by Gutman and Young (54) (1944), after crush injuries interrupting a muscle nerve in rabbits, the axone tip advanced at a rate of 4.4 mm. per day. There was a delay of not more than 5 to 7 days between arrival of the nerve fiber at the surface of the muscle fiber and its penetration into the end-plate. The end-plates on the denervated muscle fibers remained intact, even after severe atrophy, and there was no increase in the nuclei of the end-plates.

Reinnervation of muscle fibers after longer periods of atrophy, as when the nerve was crushed at a distance from the muscle, was less complete. Some of the regenerating axons failed to reach the motor end-plates, and new motor end-plates were formed where these fine fibers contacted the muscle fibers. After further section and resuturing of the nerve, reinnervation was still less complete; in this latter instance many small fibers, some sensory and sympathetic, entered the muscle and formed elaborate networks among the muscle fibers.

It was concluded that reinnervation is prejudiced: 1) by considerable atrophy, i.e., new motor end-plates are formed slowly; 2) by an abnormal pattern of reinnervation with wrong connections; and 3) failure of many muscle fibers to receive motor nerves.

After the regenerating axons reach the muscle fibers atrophy no longer progresses and some degree of muscle function is restored. A gradual recovery of muscle mass and strength of contracture follows. Usually the bulk and strength of the reinnervated muscle are less than normal because some of the nerve fibers fail to establish contact with the muscle fibers. These remain atrophic and eventually degenerate (61).

Milch (55) (1945) pointed out the drawbacks in obtaining precise measurement of muscle strength by ergographic methods to record the magnitude of flexion, extension, adduction, and abduction around a joint axis. Muscle charts can never be brought to replace clinical judgment. The decision regarding availability or desirability of any muscle transplant remains a matter for individual surgical judgment. To quote, "no amount of objective measurement of work capacity can be substituted for subjective estimate of surgeons."

When alcohol was injected into muscle, Levander (56) (1945) found an abundant development of mesenchymatous tissue around the muscle fibers. Newly-developed

muscles not infrequently grow in such a manner that they cannot be imagined as having grown from old preexisting muscle fibers. "The graft in the fully developed organism corresponds to the embryonal organizer or activator, and the newly-developed mesenchymal tissue at the place of implantation, which can be influenced by the graft in a differentiating direction, to the embryonal reactionary system." Levander is persuaded to assume that the same chemical substances are active both during the embryonal differentiation and during postfetal growth. Regeneration of tissue is considered a repetition of embryonal development.

Clark and Blomfield (1945) used a diffusible dye to ascertain the efficiency of intramuscular anastomosis in partial interruption of intramuscular vascular channels. They found that necrotic areas in devascularized muscles of rabbits are rapidly replaced by regenerated muscle fibers. In this manner the muscle is partially reconstituted (57).

Two days after the tibialis anterior muscle on both sides had been devascularized in two rabbits, the muscle was tested for devascularization. In both animals the upper third of the muscle became stained, the lower two-thirds remained unstained. Histologically the lower two-thirds showed complete dissolution and was replaced largely by strands of newly-formed fibers extending down from above. In the muscle of the opposite side, removed after three or four months, there were fibrosis and fatty infiltration of the lower two-thirds, associated with some mature muscle tissue. In the other case, the muscle showed normal mature structure. Clark concluded that new muscle elements appear to be formed entirely as outgrowth of preexisting muscle fibers (58).

In further experimental work by Clark (59) (1946), a free section of gracilis muscle was replaced in its original site in rabbits, and the animals sacrificed in 3 days to 3 weeks. At examination the transplanted muscle had undergone complete necrosis *except for a few fibers at the surface and near the margins.* Within two weeks regenerating muscle fibers were oberved in the graft in regular parallel arrangement; after weeks, well-developed cross-striations were present. Fine plasmodial outgrowth from the ends of the surrounding muscle and sarcoplasmic buds from the muscle graft were observed. The initial fibroblastic union between recipient muscle and donor graft was replaced rapidly by young muscle fibers crossing into the graft. The direction of the pathway was determined by the structure of the degenerating transplant rather than the course of the original muscle fibers. After penetration of the majority of new muscle fibers into the graft in 18 days, maturation was rapid and the usual skeletal striations were present.

Across the line of crush injury in the gracilis muscle union is evident by interweaving fibers of regenerated muscular tissue; some regenerated fibers reaching maturity, others in varying degrees of development and differentiation.

After 3 and 4 months postoperatively, in devascularized tibialis anterior muscle, the necrotic portion showed regenerated muscular tissue of approximately normal structure. When the blood supply was cut off, almost all of the endomysial cellular elements and contractile substance of muscle fibers underwent rapid dissolution. Nuclear division was by amitosis. Short segments of old established muscle fibers may give rise to regenerating fibers from both ends at the same time. Fibroblastic activity facilitated the ingrowth of regenerating fibers after ischemic necrosis. Due to bifurcation of regenerating fibers, regenerated tissue had many more fibers than the original tissue. Some of the excess fibers showed evidence of disappearing at a later regenerative stage (59).

Skeletal muscle from the region of the

knee joint and thigh of normal rats, and the human obtained mainly from amputations and radical mastectomies, was cultivated by Pogogeff and Murray (60) of Columbia University, as reported in 1946. Adult skeletal muscle from the human and the rat can regenerate and multiply *in vitro*; the multiplication being mainly amitotic. The muscle cells can exist in many different forms, the most common being spindle-shaped, round, club or rectangular fragment types, and ribbon-like forms. Macrophage-like types can be seen occasionally in the rat. Both human and rat skeletal muscle cells can contract spontaneously *in vitro*. Pogogeff and Murray concluded that cross-striations are not necessary for contraction and do not appear to be a stable component of muscle cells growing *in vitro*. The addition of calcium chloride, potassium chloride to the medium, or eserine sulfate with and without acetyl choline failed to induce or increase contraction in the concentrations used.

Weinstein and Shafiroff (61), of New York University College of Medicine (1946), placed a free autogenous muscle graft obtained from the abdominal wall or lower leg around the heart in dogs, and anchored it with cotton sutures. After 10 to 15 weeks the animals were sacrificed and the cardiac transplants examined. *The muscle grafts were successful in 2 of the 6 dogs sacrificed;* in the third dog islands of regenerating muscle were present. Total absorption of muscular elements of the graft occurred in the other dogs, with replacement by a connective-tissue layer not unlike that of fascia. In these dogs there was evidence of intrapleural pulmonary and pericardial infection. In one of the other dogs, reoperated upon after 15 weeks, when a branch of the left coronary artery was ligated, the graft had taken completely.

Examination of the muscle graft "takes" showed them to be well fixed to the myocardium, without shrinkage, but with some loss of reddish color. On section they appeared tan-yellow with white trabeculae of connective tissue. The epicardium between the graft and the myocardium was thicker than normal, with a rich vascular network. *Histologically the grafts showed normal muscle.* There was no harmful effect on cardiac function and blood circulation etc. They stated: "*It is now becoming feasible to replace necrotized and non-functioning tissue destroyed by trauma and infection with a free muscle graft.*"

As reported by Clark and Wajda (62) (1947) the tibialis anterior muscles in rabbits were devascularized, and a graph charting growth showed the average rate to be 1.2 mm. per day from the fourth to the fourteenth day, reaching 1.7 mm. per day in some instances. The growth in diameter of regenerating muscle fibers proceeds rapidly at first and in 21 days the average width of young fibers is two-thirds that of the normal fiber. After 4 months the mean diameter of the fiber in the devascularized portion of the tibialis anterior was found to be 97 per cent of the normal value. Four months after devascularization, fibers in the upper unaffected part of the tibialis anterior showed some hypertrophy. When the limb had been immobilized after devascularization of the tibialis anterior, the process of repair and regeneration was severely impaired.

The initial stages of regeneration of muscles were not retarded by tenotomy of the tibialis, but the newly-formed fibers showed more histological evidence of secondary degeneration than on the control side, and they were mingled with a greater quantity of fibroblastic tissue.

In the experimental work by Carey and his colleagues (63) (1947), the Achilles tendon of the right gastrocnemius muscle was completely severed by transverse section from the calcaneus and 3 mm. of the distal end of the tendon were excised in 150 white rats. At 24-hour intervals the morphologic

changes in the gastrocnemius muscle were compared with normally-used muscle of the left side in 5 rats over a period of 30 days. Segments of muscle and the sciatic nerve were examined histologically. In other rats tenotomy was done and two were selected for examination at 48-hour intervals.

Carey *et al.* advanced certain tentative conclusions. Normally used and innervated gastrocnemius muscle of the white rat is characterized by dark coarsely granular and light finely granular and agranular muscle fibers. The nerve fibers are generally retracted in the dark coarsely granular fiber and expanded in the light agranular fiber. The atrophy of disuse following tenotomy with the nerve supply intact is accompanied, during the first month, by progressive loss of the narrow, dark coarsely granular muscle fiber, and by depletion of its innervation. The dark granular muscle fiber is determined by the reciprocal interaction of the normally intact and attached muscle fiber with its normally-functioning innervation. During the process of atrophy after tenotomy, small and giant fusiform neurosomes are discharged from the altered nerve endings. A parallelism appears to exist between the atrophy by disuse of the tenotomized muscle and the loss of the normal discharge of neurosomes from the altered and depleted innervation of the muscle. Therefore, one factor in the atrophy appears to be the substantial loss of the discharge of neurosomes into muscle as well as the quantitative decrease of myoplasm.

Sanders (64), of the University of Oxford (1950), studied nerve homografts transplanted in rabbit's muscle. In several of the animals the regenerating muscle fibers were found to be invading the Schwann tubes of the degenerated nerves. Such invasion of nerve fibers has been found only in nerve homografts, and only after intervals sufficient to insure destruction of the original cell population of the graft. It seems that when endoneurial tubes are depleted of their cell content, they are able to serve as a support for extending regenerated muscle fibers in a manner similar to that in which the endomysial tubes of a muscle act. This invasion of the Schwann tubes of degenerated nerves by regenerating muscle fibers does not seem to occur in autografts. This indicates that muscle fibers are unable to enter the endoneurial tubes when the latter are packed with Schwann cells and macrophages.

In the experimental work by Hoffman (65) (1951) the tibial nerve of the albino rat was implanted into the pelvic quarter of the gracilis anticus muscle of the same animal. The free end of the nerve was stitched into the incision in the gracilis anticus. After 10 to 130 days examination by electrical stimulation was made for reinnervation. Nerve fibers implanted in normal muscles or muscle regions functionally deprived of innervation by separation from their innervated regions, innervate them to a small degree. Nerve fibers implanted in denervated muscles grow more actively and innervate more energetically. Activity to a similar degree is possessed by nerve fibers implanted in normal muscle adjacent to denervated muscle. This, in Hoffman's opinion, suggests involvement of the humoral mechanism. In conclusion, regenerated muscle fibers and regenerated regions of severed muscle fibers are innervated from the implant. Because of random courses of nerve fibers and the irregular innervation, direct implantation methods are less satisfactory in reinnervation than the use of old endoneurial pathways in the nerve stumps.

Andresen and his associates (66) transplanted autogenous and homogenous free muscle-fascia grafts into white rabbits and found that the basic pattern of degeneration and granulation tissue organization of both types of transplants were essentially identical. When single homogenous transplants

were made, the lymphocytic and angeitic forms of reactions were rather mild. When multiple successive homogenous transplants were made from the same donor to the same recipient,[1] acute angeitis with thrombosis supervened and a lymphocytic reaction failed to develop or persist. Multiple successive autogenous transplants, conversely, did not influence the type or degree of reaction to autogenous transplants in the same animal. There was no evidence that the presence of autogenous transplants had any influence upon the sequence of reaction to homogenous transplants or that the presence of homogenous transplants influenced the nature of the reaction to autogenous transplants in the same animal.

Cooke and Segar (67) (1952) determined the skeletal muscle composition occurring in disturbances of acid-base balance in rats. The constancy of the cation concentration of muscle seemed to depend largely upon three factors: 1) The intracellular nondiffusible anion concentration is maintained at a stable level through cell anabolism. 2) The extracellular potassium concentration is regulated within narrow limits by renal transport mechanisms. 3) The extracellular hydrogen ion concentration is maintained with little variation by respiratory and renal regulatory mechanisms operating through the buffer systems of the body. The hypothesis is postulated that intracellular sodium (entering the cell by diffusion) and intracellular hydrogen (resulting from cell metabolism) compete with each other at the cell membrane for a cation exchange system. This system transfers intracellular sodium or hydrogen outward and extracellular potassium into the cell. Cooke and Segar hold that by means of the hypothesis it is possible to predict body electrolyte composition in a variety of disturbances. Since parenteral fluids must be introduced into the extracellular space alone, the fluids administered should alter extracellular concentrations in a way that will correct deviations in intracellular composition.

[1] Second and third crop homografts.

SUMMARY COMMENT ON TRANSPLANTATION OF MUSCLE IN ANIMALS

Zielonko (1874) observed rapid necrosis of free muscle transplants in frogs with no evidence of regeneration. Gluck, however, in 1881 reported successful implantations of free muscle grafts which underwent energetic contractions when the implanted muscle was stimulated. In the enthusiasm of the day Salvia and others also reported the survival of free autogenous and even homogenous muscle grafts, which had the ability to undergo contractures when stimulated.

Magnus (1890), after careful experimental work, refuted the assumptions of Gluck and Salvia, because all of his free autogenous and homogenous muscle grafts were absorbed and replaced by fibrous tissue. Volkmann (1893) in his classic study supported the findings of Magnus, and it is now generally accepted that all free muscle grafts are absorbed and replaced by fibrous tissue.

Investigators then became interested in the cause of degeneration of free muscle grafts, and it was thought that it might be possible to influence the survival of the muscle cells by stimulating them after transfer to prevent atrophy from disuse. Kroh (1905) believed that free autogenous muscle transplants failed to survive because of the lack of normal nerve stimulations. Regular stimulation of the muscle transplant by Kroh with faradic current, however, failed to prevent degeneration and replacement of the muscle cells in free grafts.

Hildebrandt (1906) transplanted autogenous muscle grafts with interrupted blood supply but with intact motor nerve, believing that reception of the normal nerve impulses would allow the muscle transplant to

survive. The muscle cells in Hildebrandt's transplants, however, died in a matter of a few hours, *demonstrating that retention of the blood supply for muscle transplants is absolutely essential for survival of the muscle fibers or cells.*

Thus by 1906 most of the present-day beliefs regarding the behavior of free muscle grafts had been established, which may be summarized as follows: Free autogenous muscle grafts always degenerate and lose their structure as muscle with the ability to contract on stimulation. The regular stimulation of free autogenous muscle transplants with faradic current fails to prevent degeneration and replacement of the muscle fibers. The most important factor causing the death of free muscle grafts is separation of the normal blood supply. Free homogenous and heterogenous muscle grafts also fail to survive as muscle and the host tissue reaction is more marked than with autografts. Many investigators had noted gradual atrophy of muscle from disuse and hypertrophy of the same muscle when it was used or exercised. Later histologic observations confirmed clinical opinions that muscle *in situ* with intact blood supply underwent gradual but progressive atrophy when the motor nerve was severed. Thus it became generally accepted that the normal nerve impulses through the intact motor nerve are necessary for the preservation of the muscle fiber. Artificial electric stimulation to muscle *in situ* with interrupted nerve supply may delay atrophy of the muscle but it does not represent an accurate duplication of the normal nerve pattern to the muscle through an intact motor nerve. The muscle with interrupted nerve supply, therefore, will eventually degenerate in spite of the artificial faradic stimulation. Autogenous muscle flaps transplanted with adequate blood supply and intact motor nerve survive and function very well.

Of particular interest is the fact that some investigators have noted that the peripheral muscle fibers, which are in a position to receive early nourishment, have been reported to survive longer than the larger mass of centrally-located muscle cells which do not receive early adequate nourishment and therefore degenerate very early.

Two new experimental findings by different investigators have somewhat disturbed the *status quo* of accepted opinion regarding free muscle grafts. Perhaps the most important work was that of Pogogeff and Murray, who from tissue-culture studies concluded that *cross-striations are not necessary for contraction* and do not appear to be a stable component of muscle cells growing *in vitro*. Adult human and rat skeletal muscle not only can survive but actually multiply in tissue culture. The fact that adult skeletal muscle can undergo rhythmic contracture without any nerve supply whatever is rather astonishing. Admitting that the environment in tissue culture is quite different from that in the human body, still it is impressive that the skeletal muscle cell can undergo contracture without the cross-striations and other anatomical structures assumed to be necessary.

The other important recent contribution is that by Weinstein and Shapiroff (1946). These two investigators observed that some free muscle grafts transplanted in contact with heart muscle in dogs retained their structure as muscle. If these observations are substantiated by other investigators, then the heart muscle in dogs must constitute an especially favorable transplantation site where free skeletal muscle grafts can survive as such.[2] The weight of experimental evidence by others, however, very strongly indicates that muscle fibers in free skeletal muscle grafts will die in a matter of a few hours if their blood supply is completely interrupted. It is difficult to understand

[2] See Leriche and Fontaine (35).

how a blood supply for the skeletal muscle transplant could develop so quickly in a recipient cardiac muscle site. It is possible, of course, that the cardiac muscle cells of the recipient bed replaced the skeletal muscle cells in the free graft but the two types of muscle are quite different histologically, and experimental evidence is lacking that the cardiac muscle cells can give rise to skeletal muscle.

Obviously there is much to learn about this interesting and highly specialized skeletal muscle cell. The newer experimental approaches through observation of controlled tissue culture growths of adult and fetal muscle cells may radically change some of the accepted viewpoints.

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CHAPTER 29

Transplantation of Muscle in Humans

REVIEW OF LITERATURE ON HUMAN MUSCLE GRAFTS

Zenker (1) is said to have shown in 1864 that there was a regeneration of muscle fibers in typhoid fever. He believed that these fibers came from spindle-shaped cells in the sarcolemma. By nuclear divison the cells there developed a ribbon-like formation which finally became muscle fibers.

It is reported that Lexer (2) in 1867 used masseter muscle to give support and function to the region about the mouth by the intraoral approach.

In 1871 Benjamin Howard (3) reported a unique case. A major who had sustained an injury of the bones of his leg at Gettysburg in 1863, went to Europe for treatment of a large wound, 1 by 4 inches in size. As the case is related, first Howard used an autogenous skin graft. Then Hinton, who was a friend of Howard, resected a piece of biceps muscle (autogenous) from the patient's right arm, and divided it into three pieces corresponding to the excavations and deposited them on the ulcer in the same manner as skin would be treated. The next day the grafts were found to be firmly united. Centripetal cicatrization took place at a point nearest to the largest muscle graft, over which new skin was quickly applied.

In another case, the same procedure was repeated for an extensive burn on the arm. Three muscle grafts from the biceps (autogenous) of the opposite arm were inserted at an equal distance from each other in the wound. The next day all the grafts were adherent, two firmly, and the granulations had a brighter appearance. In ten days there was a narrow hard scab. Howard inferred that the muscle graft becomes "a center of new life," compelling an increased capillary supply "to converge from its own sustenance."

Helferich (4) (1883) attempted to transplant heterogenous muscle into humans. A heterograft of muscle from a dog was implanted into a defect of the biceps owing to removal of a fibrosarcoma. He held that after 3 months electrical stimulation "showed a return of normal function to the muscle." (This has been referred to previously in Chapter 25, Transplantation of Tendon in Humans.)

At a society meeting in Boston in 1895 Goldwait (5) presented a patient in whom the sartorius muscle was transplanted as a muscle flap and attached to the quadriceps extensor just above the patella. In a large number of patients with infantile paralysis in whom the thighs are involved, the sartorius and tensor vaginae femores are frequently unaffected when all of the other thigh muscles are destroyed. Without the other muscles the sartorius, from its peculiar position and attachment, produces unde-

sirable results in the use of the leg. The plan of the operation was to give the sartorius, which normally possesses considerable strength, a better mechanical attachment. In 3 out of 5 patients on whom the procedure was carried out there was marked improvement; in two the result was disappointing. The strength of this muscle increased until it was able to do the work of the quadriceps that would be required in walking or standing.

Ribbert (6) (1898) held that successful transplantations are possible if care is taken to provide nourishment and quick application of functional stimulus.

Only a short time later we find reference to a practical application of Ribbert's recognition of the need to provide nourishment. Abrashanoff (7) (1900) was the pioneer in the use of a pedicled muscle flap in bronchial fistula.

In a case of facial paralysis of 3 months' duration after operation for suppuration in the mastoid process, Gersuny (8) in 1906 sectioned the musculus orbicularis oris in the midline and sutured the unparalyzed half at the corner of the mouth on the involved side to the paralyzed part of the muscle. The paralysis was completely corrected after a relatively short time. In a second case of paralysis of the right deltoid muscle the insertion of the trapezius muscle was freed at the acromion and the lateral part of the spina scapulae. The insertion of the deltoid muscle in the scapula was cut through; the deltoid was pushed far under the separated trapezius and sutured to it. Gradual improvement resulted in good function, with normal contracture of the deltoid.

In a case of a 5-year-old girl, demonstrated by Deutschländer (9) in 1909, a part of the healthy gluteus maximus was transplanted as a flap to replace the completely paralyzed gluteus medius and minimus. The transplanted muscle segment was completely maintained as capable of function and 10 weeks postoperatively it functioned well. The shaky hip joint became fixed, the gait was more certain, and the Trendelenburg phenomenon formerly present vanished.

Nélaton (10) (1909) recommended muscle transplantation in bone cavities caused by resection in osteomyelitis. This procedure resulted in complete filling in with tissue. Jianu (11) (1909) and Edén (12) (1911) reported the use of the masseter muscle as a muscle flap for support and function about the mouth by the extraoral approach.

In a case of removal of a tumor of the fifth rib, and in another case of chrondrosarcoma of the sternum and left fifth costal cartilage, Robson (13) (1911) transplanted a flap taken from the pectoral muscle to cover a removed portion of the pericardial wall. Although the operation resulted in immediate success in both instances, Robson did not recommend repetition of the procedure because of the later unfavorable progress.

Unger (14) (1910) and Kocher (15) (1912) used free muscle transplants, laying them on the bleeding area in cranial hemorrhage in order to effect hemostasis by thrombokinesis.

In the Eighty-fourth Meeting of the German *Naturforscher und der Aerzte,* Goebel (16) in 1912 presented a 5-year-old boy in whom *a free muscle transplantation had been carried out successfuly.* An ischemic contracture of the flexor muscle of the forearm was associated with an extension fracture of the humerus. The muscles were freed from their scar adhesions and separated at the passage of the muscle into the tendon. The upper end of the right sartorius was implanted as a free graft into the gap arising through extension of the finger in place of the flexor profundus. The points of the external oblique muscle corresponding to the tenth intercostal nerve were implanted as a free graft into the flexor sublimis. The nerves

belonging to these muscle sections remaining in connection with them were implanted in different places in the median nerve. The scar in the fascia of the forearm was replaced by a piece of fascia lata. *After seven months function was normal; the fingers could be completely extended and flexed with power.*

In perforating wounds of the left ventricle with torn heart muscles in two patients, Läwen (17) (1912) used free muscle plasty, with immediate but not ultimate good result. A piece of free M. pectoralis major was laid on the heart wound and made firm with some loose silk threads drawn together. After the death of the first patient 5 days postoperatively, examination showed the transplanted muscle to be firmly united to the heart muscle, to contain blood-filled vessels, and to be grown to the pericardium lightly. After the death of the second patient on the thirty-first day postoperatively, the muscle piece was changed to a yellowish mass firmly united to the heart muscle and pericardium.

A successful replantation of a forearm was reported by Jianu (18) in 1913. The lower third of the patient's forearm was displaced and connected to the body only by a skin flap which contained a vein. Following the replantation union of the bone and muscle, vessels and nerves took place. Three years postoperatively the hand was maintained, with many disturbances in sensation, and motor and vasomotor responses, which were regressing.

The view was held by Schulz (19) (1918) that musculature is in no way suitable for free transplantation, because the young tissue, cut off from functional and nervous influences, cannot endure but succumbs to necrosis.

In 2 of the 3 patients on whom an operation on the lower leg had to be performed, Eden (20) (1919) removed portions of healthy musculature from the extensor digitorum communis containing a piece of fascia and implanted them in their original locations. In the third patient healthy pieces from the anterior tibialis were similarly implanted. After different intervals muscle pieces were examined at a second operation. The muscle fibers were distintegrated and were replaced by connective tissue. Retained or proliferated muscle elements were present at the margin of the transplant, but these, Eden thought, could derive from the matrix. Free transplanted muscle tissue, he believed, is always destroyed and is finally replaced by connective tissue. The adhesion of the transplant to the wound surface is the essential factor in hemostasis.

In a patient with loss of the whole soft part of the nose by injury, extending to the pyriform aperture, Schloffer (21) (1919) used a muscle transplant. This was a pedicled flap from the right side of the forehead including all soft parts, except periosteum, and a piece of frontalis muscle. Two small plates from rib cartilage were sutured to the flap. The cosmetic result was tolerable.

Wullstein (22) (1922) considered the following conditions as indications for muscle flap transplantation: spinal paralysis, paralyses after apoplexy, traumatic changes, spastic paralyses, progressive muscle atrophy, multiple sclerosis, scoliosis, congenital deformities of the foot, ischemic contractures, and genu recurvatum.

The suggestion was made by Starr (23) (1922) that viable pedicled muscle flap be used in deep rigid-walled bone defect caused by osteomyelitis. Wangensteen (24) (1925) advocated the insertion of a flap of muscle from the intercostal bundle to aid in plugging a bronchial fistula.

Kisch (25) (1932) used a temporal pedicled muscle flap to fill a cavity after mastoid operation. He considered that the graft must be cut and mobilized completely so that it lies flaccid in the bony cavity in order to obtain good results. A certain

amount of fibers are separated, a good connection being left to the main mass of muscle. Kisch employed this procedure in various types of mastoiditis (after radical mastoid operation, for a cavity after acute mastoiditis and operation in chronic mastoiditis in children, mastoid fistula) and to stop hemorrhage from an injured lateral sinus.

Garlock (26) (1934) described the operative repair of bronchial fistula through an adequate incision and the application of a pedicled muscle flap taken from neighboring muscle tissue (from the edge of the pectoral muscle). In another patient a flap was fashioned from chest musculature. The end of the flap is inserted into the bronchus, and the sides of the flap are sutured to the tissues in the vicinity. This procedure was uniformly successful in Garlock's hands, and he believed the operation has a wide field of usefulness.

Roskin (27) (1934) studied pieces of myosarcoma furnished by the *Institute de Radium* of Paris in five cases. Based on their experimental material and the literature, he affirmed the existence of a great morphologic plasticity of polymorphous myofibrils which are formed in the myomatous cells. The different parts of the same fibril can have a smooth structure as well as a striated one. Some parts of the cell form fibrils, whereas others do not produce them. The plasma gives rise simultaneously to smooth and striated fibrils in the muscular cell. The processes of maturity can follow a normal or abnormal course, or give smooth or striated fibers. The histogenesis, regeneration, and development of the myomatous cells *in vitro*, and the blastomatous growth, each is characterized by a histologic symptom-complex which is suitable to it. Cytologically, these processes are not identical; each unfolding according to its own pattern. Roskin concluded that the simple return to the embryonic stages is impossible, that embryogenesis is not reproduced, but that the highly specialized cells can, at the same time, be highly plastic and manifest not only certain phenomena suitable to the myoblasts, but can acquire new histophysiologic qualities as established in the formation of intra- and extracellular (precollagenous) fibrils.

Vinke (28) (1934) designed an instrument—the myokinesismeter—for accurately recording muscular action in the gait. The record obtained shows the time and the extent of muscular contractions as compared with various phases of gait. In conjunction with the myokinesismeter, a sandal with special electrical switches was constructed to determine the various phases of gait. Vinke believed that his method is useful in making the proper selection of muscles for transplantation, because there is variation in muscular contractions. The choice of the distribution of muscular power by transplantation of a tendon should be made by kinetic investigation, thus determining the absence of individual muscular deficiency. The evidence revealed that the transplanted muscles retain their original or nearly original contraction wave after transplantation. The study of transplantation of muscles in spastic and infantile paralysis showed that the biceps femoris can be transplanted to the quadriceps with good clinical results.

Beck (29) in 1935 conceived the idea of improving the circulation of the heart muscle by throwing a flap of pectoral muscle over the depleted area of cardiac muscle.

Sheehan (30) (1935) regarded the muscle flap as a muscle-nerve graft. Procedures were designed to bring to paralyzed areas about the mouth and nose effective reanimation from the temporal muscle. The basic requirement in unilateral facial paralysis is to counterbalance the pull by live muscles on one side of the mouth upon the inert muscles in and about the oral orbicularis on the affected side. The terminal strands of a

fan-shaped end of the pedicled strip are brought into approximation with and sutured to the muscles to be reanimated: the zygomatic, the levator and depressor alae nasi, the labii superioris at its junction with the caput angularis, and any other muscles related to the orbicularis oris as well as that muscle itself and its extensions.

In four patients with lymphedema of the arm following radical breast amputation Reinhoff (31) (1937) used a fan-shaped muscle flap cut out of the latissimus dorsi so as to include the subscapular nerve, artery, and vein. The free edge of the muscle formed the future upper margin of the pedicled flap. The latissimus dorsi was split along the course of its fibers up to within a few centimeters of its insertion. The edges of the flap were sutured to the remaining remnants of the origin of the pectoralis major from the clavicle and the sternum, and also to the internal and external intercostal muscles of the first and second interspaces. The wound healed perfectly in each instance. Reinhoff suggested the employment of pedicled muscle flap for the relief of lymphedema elsewhere.

Yount (32) (1938) reported on an operative procedure devised for use in a particular group of patients with a fairly uniform type of quadriceps paralysis and a similar residual power in the extremity. In the 16 patients operated upon, knee flexion and hip flexion contractures were present. In 8 patients the biceps muscle was transplanted along with the iliotibial band. A tube constructed from the inner lip of the cut fascia lata is made by folding it upon itself toward the mesial aspect of the limb. The tube is placed so it lies laterally and then gradually extends obliquely toward the middle as it approaches a point about 3 inches above the patella. The transplant of tensor fasciae latae enters the tube at the top, but a separate opening in the tube is made for the biceps muscle. After emergence from the tunnel the biceps is stitched to the fascial transplant. Both these structures are then sutured to the quadriceps tendon. The fascial transplant is divided into two parts at the end and is embedded in two holes in the patella.

Stephens and Benteen (33) (1938) had an opportunity to study at necropsy a pedicled muscle graft which had served to close the remains of a lung abscess cavity and several bronchial openings two years previously. The graft could still be grossly distinguished from the neighboring lung parenchyma. In their opinion, the muscle had remained viable and had filled the cavity in the left lower lobe. For the most part the striated muscle was replaced by fibrous tissue, but histologically many striations were still to be seen. Many large blood vessels, both in the substance of the graft and running along the surface of the graft, connected the visceral surface of the lung to the chest wall. Stephens and Benteen regarded pedicled muscle grafts as serviceable transplants; they retain their bulk for a long time, and resist ferments of secretion and suppuration.

After treatment and drainage in chronic emphysema the residual cavity following expansion of the lung was filled with muscle flaps, as reported by Carter (34) (1938). A flap of sacrospinalis muscle was implanted into the apical portion, a flap of trapezius and latissimus dorsi muscles into the subscapular portion, and these muscles in part with intercostal muscles into the remainder of the cavity. Carter also used muscle flap in the closure of bronchial fistulas, of extrapleural cavities resulting from the excision of cold abscess of the chest wall, in cure of chronic lung abscess, and in secondary closure of infected thoracoplasty wounds in which an extrapleural cavity remained beneath the scapula.

A long pedicled muscle flap from the paravertebral muscle was used by Coryllos and Ornstein (35) (1938) to fill very large tuberculous cavities in the lungs in 12 patients.

The immediate results were satisfactory. In all patients except 2 the wound closed by primary healing. There was secondary opening between 10 and 20 days postoperatively. Pathologic study was made of specimens taken from patients who died from 3 weeks to 4 months after operation. The muscle flap became actively tuberculous during the first 3 weeks. At the autopsy of the patient who died 4 months postoperatively, the flap was *in situ* and completely adherent to the walls of the cavity, and it was transformed into fibrous tissue. In their hands the muscle flap gave 50 per cent negative sputum but they regard it as a procedure still fraught with dangerous complications.

Crafoord and Linton (36) of Stockholm (1940) used an autogenous pedicled muscle flap from the latissimus dorsi in bronchial fistulas after pulmonary abscess in 16 operations during a period of 5 years. The time between the opening of the abscess and the plastic operation varied between 1½ and 20 months. With one exception all cases resulted in relatively rapid healing. Crafoord and Linton considered it an excellent therapeutic method in residual cavities. They stated that it is uncertain what happens to the flap after operation.

Countryman (37) (1942) reported on successful use of free grafts of autogenous rectus muscle from the incision as tamponade of liver wounds in 8 patients. In two cases autopsy examination showed rapid healing in the liver repair without liquefaction of the transplant and apparent regeneration in some of the muscle fibers.

It appears that regularly-repeated galvanic stimulation delays the loss of weight of denervated rabbit muscle as compared with a control, according to Gutmann and Gutmann (38) (1942). The process of atrophy, however, was not prevented, and after 4 weeks the treated muscles had lost approximately as much weight as the controls. The speed of recovery of function after reinnervation was not significantly changed.

Prudente (39) (1944) transported free muscle grafts through skin tubes for transplantation to repair defect of the mouth and cheek. In the fourth patient cancer of the floor of the mouth involved the jaw and lower lip. Anatomical and functional reconstruction resulted in 2 patients, and restoration is still taking place in 2. Healing of a muscular skin flap is always by first intention because it concerns union between tissues of the same nature. As Prudente described the process, when the muscle strip is isolated before its transplantation, it is later transformed into fibrous tissue; but after that transplantation, when it lies in approximation to healthy muscles of the new region, it recovers its normal appearance, corresponding to a true rehabilitation of the tissue. *After a few months the grafted muscle recovers its function,*[1] probably due to nerve penetration from neighboring muscles, and the patient is able to perform every movement with real contraction of the grafted muscle. Histologic examination shows perfectly normal structure of the grafted muscle.

Bowden and Gutman (40) (1944) have observed some identifiable muscle tissue in biopsy specimens of human muscle which had been denervated for as long a period as 26 years.

As stated by Sterling Bunnell (41), signs of recovery of sensation in muscle usually begin 5 months after suture of the nerve, except in the hand, where they may be seen in the first month. After return of sensation over the whole limb to its tip, the quality of the sensation is at first far from normal, for paresthesia is felt in response to the various stimuli. This may persist in lessening degree for 1 to 3 years, at which time the quality of sensation usually approaches the normal.

Voluntary motion may not appear until 6 or 8 months after nerve repair but when

[1] Prudente's findings do not agree with those of other modern investigators.

the first muscle is not far from the nerve juncture, as, for instance, after suture of the posterior interosseous nerve, its return has been observed by Bunnell as early as 3 months postoperatively. As a rule each muscle becomes innervated consecutively, starting with the most proximal ones and ending with the distal ones. Extensors or flexors of the wrist generally show movement first, then the extensors or flexors of the fingers and finally the extensors and flexors of the thumb. The intrinsic muscles in the hand are usually the last to obtain voluntary movement.

To overcome symptoms of "aseptic necrosis" and painful osteoarthritis of the hip secondary to old dislocations and other conditions, Venable and Stuck (42) (1945) cut a slot in the neck of the femur from the base of the head to the trochanteric region. An autogenous flap from the medial half of the vastus lateralis muscle is dissected free and transplanted into the slot, and anchored through the capsule. This procedure results in rapid relief of pain from the "decompression" of the neck. The muscle flap serves to keep the opening in the bone from closing, and soon an auxiliary blood supply is directed into the head. Postoperative roentgenograms have frequently demonstrated increased proliferation of the bone and partial regeneration in the head. Twenty-six out of 27 patients in whom muscle transplantation was carried out gained marked relief, which has lasted as long as 2 years since the first operations.

In a critical review of the work done on animals and clinically, Sperry (43) (1945) found that the evidence reveals little support for even moderate assertions concerning central nervous reintegration of muscle. In his view, various types of compensatory adjustment on the part of the intact system, along with local mechanical and trophic changes in affected parts, have produced an effect erroneously interpreted to be the product of extreme revision in the central synaptic associations of the affected peripheral nerves. There is no convincing support for the idea of recovery by instantaneous dynamic reorganization. Sperry considers that a selective readaptation of nerve-fiber connections may continue to take place after regeneration is in the main completed. Reeducation adjustments are not accomplished in the primary motor or sensory nuclei but involve the higher association centers.

Begg (44) of Johannesburg (1946) reported on his experience with the interposition of a slice of free muscle between the cut sides of severed renal parenchyma in nephrotomy, partial resection, or dividing the isthmus of a horseshoe kidney. He reenforces the capsular sutures by another muscle strip firmly bound down to the incised margin. A well-fitting pad of free muscle is pressed against the cut surface of a heminephrectomy for postoperative bleeding. Bleeding from extrapelvic vessels is controlled by a small piece of free muscle or by including it in a loop of a Lembert suture taken through the capsule. A strip of freshly cut muscle is laid on an incision in the ureter or pelvis, overlapping it a little at each end. Suture of a bladder incision is satisfactorily reinforced with free muscle. The pyramidalis is used to separate two surfaces of vesical and vaginal walls by interposition of free muscle in vesicovaginal fistula. To quote, "the muscle incorporates itself in the surrounding tissues, forming a strong partition between the two hollow organs." After free dissection the whole of the left pyramidalis is interposed between the rectal and urethral suture lines in recto-urethral fistula. Begg points out the necessity for the graft being pressed directly on the surface or incision to be healed without intervention of any catgut, and sutures must be planned accordingly.

Adams (45) (1946) held that the transplantation of the masseter muscle gives a more natural effect than that of the tempora-

lis muscle for giving motion to the lower half of the face, and is ideally located for the purpose. The temporalis muscle is best suited to aid in restoring motion and to give support to the eyelids. To obtain a satisfactory result for reanimation of a paralyzed eyebrow and forehead region, Adams recommended shifting a flap of the lower insertion of the frontalis muscle just across the midline of the forehead. In the complete procedure three muscles are employed in an effort to give as much motion as possible to the paralyzed side of the face, the fibers being separated vertically.

In some cases of chronic osteomyelitis Prigge (46) (1946) obliterated the defect with muscle transplant. He selected the muscle in such a manner that the innervation and blood supply of the donor muscle and the blood supply of the pedicle would not be jeopardized. His object was to insure a minimum interference with function of the part and a viable pedicle. He obtained good results in 43 of 44 foci of infection in 42 patients treated by this method. When the defect could not be obliterated by muscle transplant, autograft from the iliac crest in thin strips was used for filling.

An anatomical observation by Keith that the third pectoral muscle—the pectoralis externus—is commonly fused with and forms part of the pectoralis major, impressed Clark. On the basis of this, he thought it reasonable to expect the outer portion of the pectoralis major to have a nerve supply separate from the remainder. Thus it would be possible to construct a viable transplant from this part of the muscle. Clark (47) (1946) gave an interesting report on the case of a German soldier who sustained a shell wound of the left upper arm, laceration of the biceps and coracobrachialis muscles with musculospinal nerve palsy. Five and a half months later a tendon transplant was employed to restore extension of the wrist and digits, with a satisfactory result. In an operation on the arm at the Leeds General Infirmary, Clark detached the origin of the pectoral muscle from the anterior surface of the sixth rib, together with its covering fascia, to provide tissue. After separation of the pectoral fibers along a line parallel to the muscle's axillary border a transplant was isolated. As the separate part of the muscle was turned upward, the loose fascia under it was stretched and the nerve—a main terminal branch of the lateral thoracic nerve supplying the lateral part of the great pectoral muscle—assumed an approximately horizontal position and was preserved. A subcutaneous tunnel was made communicating with the upper end of an incision in the arm where the pectoralis muscle was exposed. The pectoral transplant was threaded through this tunnel. The tendon stump was lopped through the distal end of the transplanted muscle and sutured to itself. Six weeks postoperatively full extension at the elbow was short only by 20 degrees and active flexion was possible to 70 degress. The substituted muscle became three times its normal size and had action and appearance closely simulating that of a normal biceps muscle.

For a residual pleuropulmonary cavity following external drainage of lung abscess, associated with bronchial fistula, Prioleau (48) (1946) used as filling and for closure a pedicled muscle flap. The cavity is prepared by adequate drainage, unroofing and thoracoplastic measures, and then is obliterated by implanting the pedicled flap. Two cases are reported in which the results were excellent and satisfactory, respectively.

Bowden (49) (1947) has frequently found new formation of smooth muscle fibers in the lungs in long-standing bronchiectasis. In one patient with brown induration accompanying severe rheumatic heart disease, microscopic examination of sections showed myriads of small smooth muscle bundles present in the thickened interalveolar walls.

Bowden gave illustrations of his findings of muscular scar formation in the lung: extensive proliferation of smooth muscle tissue, thickened interalveolar walls containing a large amount of smooth muscle tissue; a few muscle bundles, and new formation of smooth muscle embedded in fibrous tissue. Smooth muscle makes its appearance away from its normal sites.

Owens (50) (1947) considers that the method of choice for correction of facial paralysis is one designed to give reanimation as well as support to the involved side of the face. For this purpose fascial strips are incorporated in the substance of the upper and lower lips and in the tissues at the angle of the mouth and then anchored in the substance of the masseter muscle. Paralyzed muscles are thus connected with a functioning masseter muscle. In Owen's experience this procedure gave satisfactory results, providing support as well as a fair amount of reanimation to the relaxed tissues. All important structures are under direct visualization in this procedure.

As pointed out by Adams (51) (1948), paralysis of the facial nerve involves loss of function of 11 major and 9 minor muscles of expression about the eyes and mouth. Consequently, the larger the number of muscles which can be brought to replace the paralyzed structure, the better will be the function. Adams described a method in which pedicled flaps from the masseter, the temporalis, and the frontalis muscles are transferred to give support and motion to the corner of the mouth, the eyelids and the brow respectively. In comparison with the use of only one muscle, this method gives better function and more normal direction of pull. The point of insertion of each muscle is pedunculated. He held that the factors in success are whether the pedicles are made sufficiently large enough to provide support to the paralyzed area, whether the nerve supply is protected in separating the muscles, and whether the flaps are attached under the proper amount of tension and without angulation.

Mallah (52) of Cairo (1948) resected a rib in a 13-year-old boy for drainage of fluid from the lung. The patient was again operated upon for abscess of the lung, and then for closure of a fistula, entailing partial resection of the ribs around the fistula, with dissection of the fistula itself. Because of the presence of a persistent fistula in the scapular line, there was an external opening, 2 inches in diameter. A pedicled piece of muscle was turned upward from the sacrospinalis muscle, plugged in the cavity, and sutured at the corners of the cavity. The wound healed by primary intention, and the patient was considered cured.

Thiemeyer (53) (1950) believes that a muscle flap may be applied in any area of chronic osteomyelitis where an adequate supply of muscle is available which can be transplanted without jeopardizing the function of the part. The base of the flap must at least be as wide as the length and preferably twice as wide and it must be defined to transmit a blood supply. The flap is laid in the defect and held by catgut sutures. Thiemeyer considers a mixture of dried plasma and penicillin effective in filling any remaining cavity. The results in the three cases of osteomyelitis reported were fair or good, and in one the prognosis was excellent after the application of muscle flaps.

Harmon (54) (1950) reported on 5 cases in which the patients, who had had poliomyelitis, were operated for surgical reconstruction of a paralytic shoulder by muscle transplantation. In the first instance the posterior third of the deltoid was transplanted to the anterior site. In the second, the proximal portion of the pectoralis major was transferred to the acromion region, the origin of the posterior deltoid remnant was shifted to the tip of the acromion and the outer fourth of the clavicle; the origins

of the long head of the triceps and the short head of the biceps were transferred to the tip of the acromion. In the second stage a portion of the pectoralis major was transferred to the region of the deltoid tubercle. The insertions of the latissimus dorsi and teres major over the humerus were transferred to the lateral margin of the bicipital groove. In the third patient the origin of the posterior deltoid and the long head of the triceps at its origin were transplanted. In the other patients multiple transplantations were carried out in one stage. The outcome in all cases was good. When the posterior deltoid remains available for anterior transplantation and when some or all of the axillary muscles are functioning, the chances of a successful result, as viewed by Harmon, are better.

In a pharyngoplasty Hynes (55) (1950) raises a mucomuscular flap on each lateral pharyngeal wall consisting of the salpingopharyngeus muscle and its overlying mucosa and transplants them upwards and inwards until they lie in a transverse mucosal defect produced across the posterior wall of the nasopharynx. After a period of a month or more the cleft palate is repaired. Hynes does not advise this procedure in patients under 10 years of age. This type of operation was carried out on 12 patients, and in 8 of these the palate was repaired at a later stage. The absent salpingopharyngei are adequately compensated by the superior constrictor palatopharyngei. Additionally, the operation itself produces marked lateral narrowing of the pharynx by the closure of the secondary defect in the lateral pharyngeal walls. Hynes reported an improvement in speech in every completed case.

Peer and Walker (56) (1951) buried 13 free autogenous skeletal muscle grafts in humans under two conditions of transplantation, namely: grafts in contact with unlike tissue and grafts in contact with like tissue (muscle). The transplants were removed at intervals of a few days to 7 months after transfer and examined grossly and microscopically. They observed that the muscle fibers in the free muscle grafts degenerated very rapidly regardless of the host site (like or unlike tissue). The fibroblasts in the connective tissue around the muscle cells, however, survived as living cells and the graft structure was changed into fibrous tissue due largely to proliferation of these surviving fibroblasts in the graft rather than to infiltrating fibroblasts from the host tissues. The blood vessels in the muscle grafts with their endothelial cell lining also tended to survive, and early circulation was established through end-to-end anastomoses between graft and host blood vessels. The muscle grafts lost their characteristic structure as muscle and at about one month after transfer no muscle cells could be identified as such. The fibrous tissue which replaced the muscle graft was greatly reduced in size compared with the bulk of the original muscle graft. Peer and Walker emphasize that muscle cells or fibers are probably much longer than is generally supposed, and in some instances they may extend for the full length of the muscle. A small free muscle graft, therefore, may consist partly or wholly of segments of muscle cells rather than of complete cell entities. When muscle cells are in contact with muscle, the cut ends of the host muscle fibers attempt to replace the fibers in the graft but this is prevented by proliferation of fibroblasts in the stroma of the graft. Peer postulates a cell-survival theory based on his study of various free autogenous tissue grafts in humans. "The cells in free autogenous human grafts tend to survive when they are transplanted as complete cell entities in favorable transplantation sites." He notes that muscle cells may be the one exception to this theory, since the muscle cells in free grafts will die in a matter of hours when deprived of their blood supply. Single human muscle cells

and small groups of cells in tissue culture, however, receive adequate exchange and both survive and proliferate.

Hynes (57) (1953) reported on the results of the pharyngoplasty by muscle transplantation in operated cleft-palate patients with anterior shortening and nasal speech defects as described by him in 1950. In 22 of 36 patients followed up for a year or more after operative treatment, the posterior pharyngeal ridge produced by the pharyngoplasty remained static and non-contractile despite the fact that the muscle was present in the flaps at the time of operation. This, Hynes considers the result of operative damage to the muscle or its nerve supply. Perfect speech will result provided the palate is mobile. In the remaining 15 patients the postpharyngeal ridge remained actively contractile owing to survival of the muscle fibers in its substance. An actively contractile muscle sphincter had been formed around the palatopharyngeal isthmus, so it contracted smoothly and well with minimum reeducation by speech therapy, as its anterior and posterior elements normally contract together. During the past year larger amounts have been included in the lateral pharyngeal flaps, and actively moving posterior pharyngeal ridges have been produced in almost every patient.

As observed by Adams, Denny-Browne and Pearson (58) (1953), after section of either the muscular nerves or the motor nerve roots there is a perceptible reduction in diameter of muscle fibers at the end of a month and the nuclei become more scattered. The striations remain, however. At the end of two months the decrease in diameter of the muscle fibers is far advanced, most muscle fibers being reduced to one-third or one-half of their normal diameter, but striations are still present. At four months after denervation atrophy is progressing less rapidly. From this period on, by almost imperceptible degrees, the muscle cells shrink to small tubes containing a ro of nuclei and some poorly staining fibril In the end only a string of darkly-stainin rounded muscle nuclei remains. Adams ar his colleagues note that the small musc fibers of the muscle spindles seem pec liarly resistant to the process of degener tion atrophy.

SUMMARY COMMENT ON MUSCLI GRAFTS IN HUMANS

At the present time, from the report evidence, one can safely state that the mu cle cells in free muscle grafts invariably d are replaced by fibrous tissue, and fail resume their normal active function. Son investigators report that a few of the p ripheral muscle cells, which are favorab situated for immediate nourishment ar exchange, may survive as muscle. T author has not observed the survival such peripherally-located muscle cells in ar of his own free human skeletal muscle graf in humans, but it is theoretically possible.

It is usually stated that free muscle graf

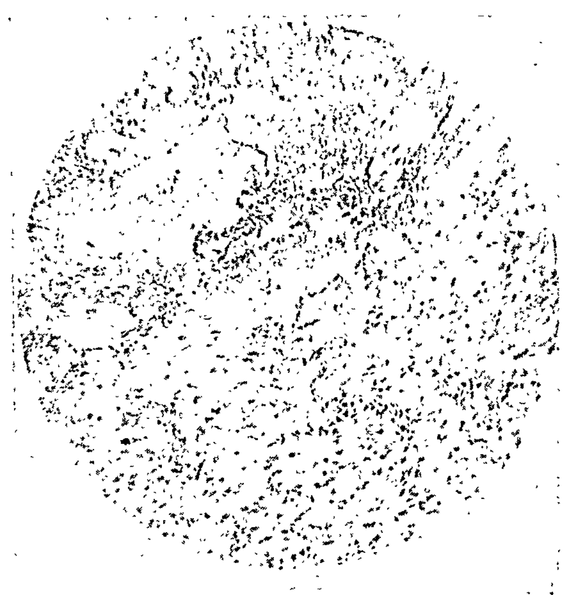


FIG. 141. Free autogenous human muscle gra in contact with muscle for 18 days. The nuclei the skeletal muscle cells are disintegrating and t muscle cells have lost their striated appearan The fibroblast cells in the connective-tissue stror have proliferated.

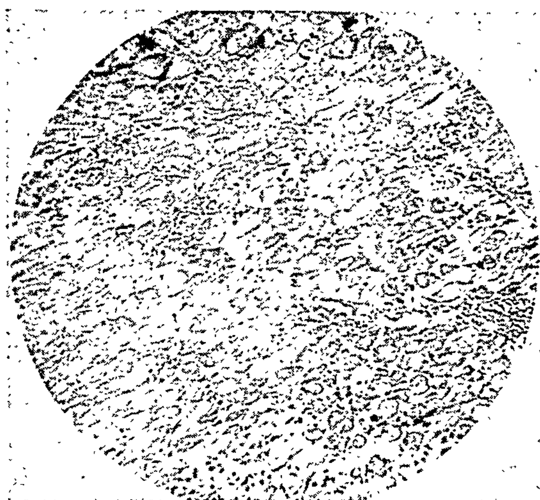


Fig. 142. Free autogenous human muscle graft in contact with muscle for 28 days. Nuclei of muscle cells have entirely disappeared. Connective-tissue cells in stroma of graft have survived as living cells which will eventually replace the disintegrating muscle cells. Fibroblasts from the surrounding host tissue participate in the replacement to a limited extent. The blood vessels in free muscle grafts appear to survive but circulation is usually more delayed than that which occurs in other vascular free grafts. The circulation was established in this series between the 4th and 5th day largely through anastomosis between host and graft blood vessels. The endothelial cells lining blood vessels in the muscle grafts appeared to survive.

are replaced by fibrous tissue, and this replacement is assumed to occur through the activity of the host tissue cells. That is to say, the free muscle graft is entirely absorbed and replaced by infiltrating fibroblasts from the host tissues. A careful study of free human skeletal muscle grafts by the author, however, indicated that only the muscle cells in the grafts degenerate and die. *The fibroblasts in the connective-tissue stroma between the muscle cells survive as living cells,* and it is these entities which proliferate and largely replace the dead muscle fibers. Thus, the fibroblasts in the stroma of free muscle grafts resemble the endoneurial fibroblast cells in free nerve grafts, which also survive and proliferate.

Although some host capillary ingrowth does occur in free muscle grafts, the original vascular system of the grafts tends to survive, with its endothelial cell lining. Early circulation for the muscle grafts (in 4 to 5 days) is made possible through end-to-end anastomoses between host and graft blood vessels.

Since the end-result of all free skeletal muscle grafts in humans is fibrous tissue, the clinical use of the transplants is very limited. Thus, Harold Neuhof's statement

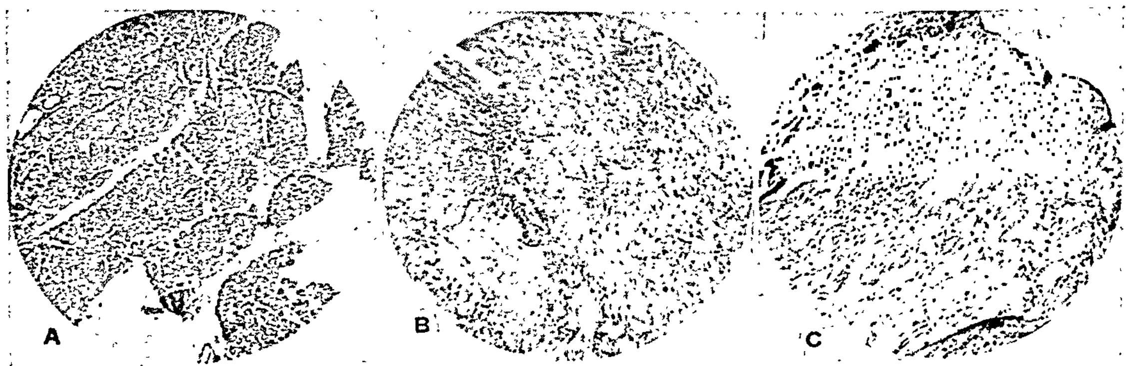


Fig. 143. A. Muscle graft (autogenous human) in fat 8 days after burial. Nuclei of muscle cells have degenerated.

B. Muscle graft in fat 12 days after burial. Complete breakdown of muscle cells. Fibroblasts in supporting framework survive.

C. Site of muscle graft 2 months after burial. Muscle cells have been completely replaced by fibrous tissue.

Drawings Indicating Usual Behavior of Free Muscle Grafts and Muscle Flaps in Man

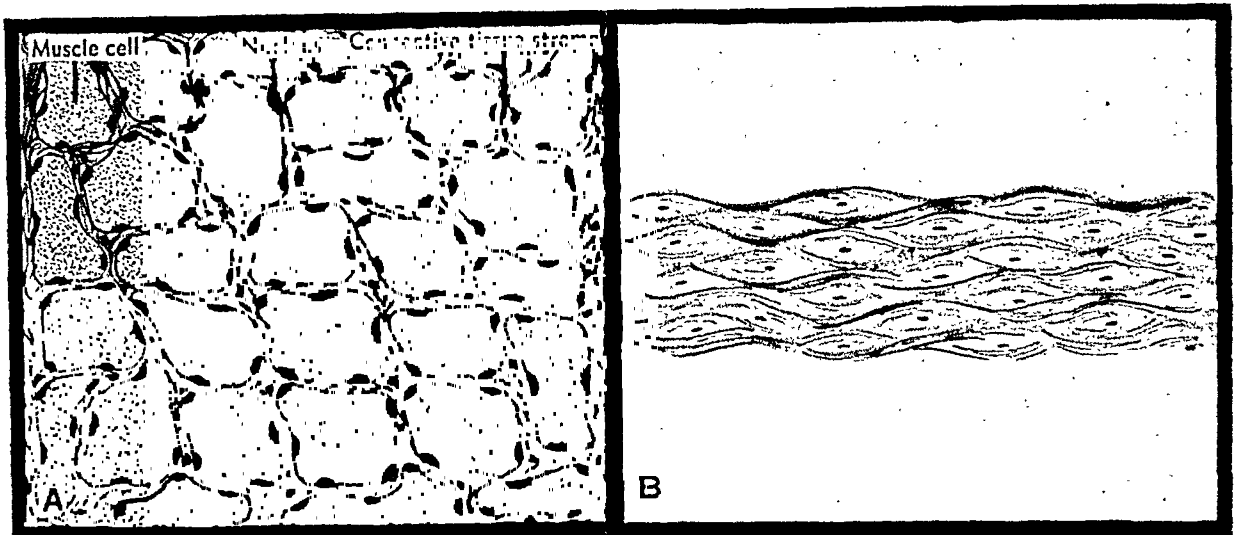


Fig. 144. A. Autogenous human skeletal muscle grafts in contact with muscle or fat. B. Grafts replaced by fibrous connective tissue. The muscle cells degenerate and die but the fibroblasts in the connective tissue stroma appear to survive.

(59) regarding the surgical indications for free muscle grafts in 1923 still applies in 1955.

The original purpose in muscle transplantation was the replacement of muscles that had become useless because of disease or injury. This object has not been attained. There is no indication for muscle grafting of small muscle defects, adequate repair occurring after suture. The free transplantation of muscle is futile for the replacement of larger defects, degeneration and fibrosis of the graft being the end-result.

The possibility of successfully transplanting a free muscle graft with severed nerve, and, in addition, *severed blood supply* appears remote in the light of our present knowledge. Muscle will degenerate in a short

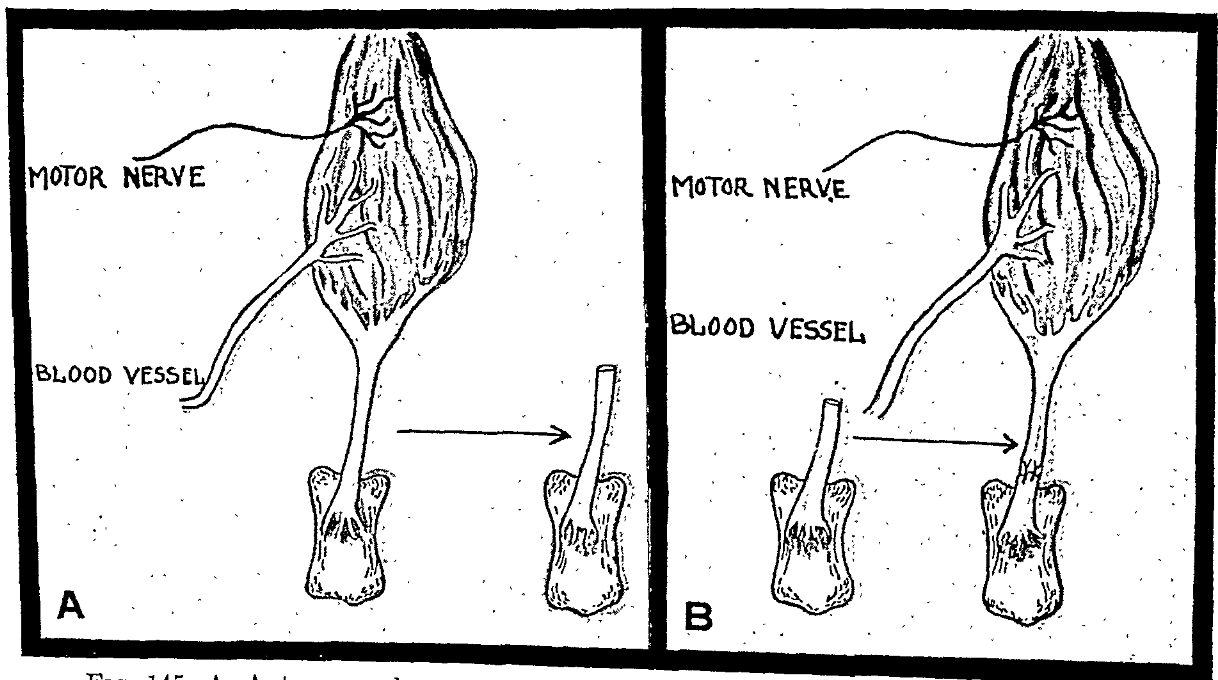


Fig. 145. A. Autogenous human skeletal muscle flaps with motor nerve and blood vessels. B. Flaps survive as muscle.

time if it is deprived of its blood supply (a matter of a few hours). Individual adult human skeletal muscle cells and small groups of these cells have been kept alive in tissue cultures for a year and a half, of course without any innervation whatsoever, and these cells grow and undergo rhythmic contractures. This is astonishing in view of the fact that muscle *in situ* with uninterrupted blood supply will degenerate when its motor nerve is cut. Obviously there is much to be learned about this interesting problem. The fluid medium surrounding individual cells in tissue culture appears to provide adequate nourishment and elimination of waste products, whereas in free muscle transplants in the human body only the surface cells are in a favorable location for immediate exchange.

Despite the contradictory evidence regarding the fate of skeletal muscle in tissue culture and in the human body, the problem of successful free muscle grafting does not appear insoluble. A beginning would be made if it were possible to cut the motor nerve and keep the muscle cells from degenerating by duplicating the normal efferent-nerve wave pattern.

In free muscle grafts there is the additional problem of providing immediate and adequate circulatory exchange for the muscle cells or putting them in a state of suspended animation until this adequate circulation is established. Another consideration for successful free muscle transplantation is the possibility that muscle cells may be much longer than they are assumed to be. Thus, a section of a muscle may be largely composed of segments of cells rather than of complete cell entities. These segments of cells may fail to survive, and the waste products formed by their degeneration may

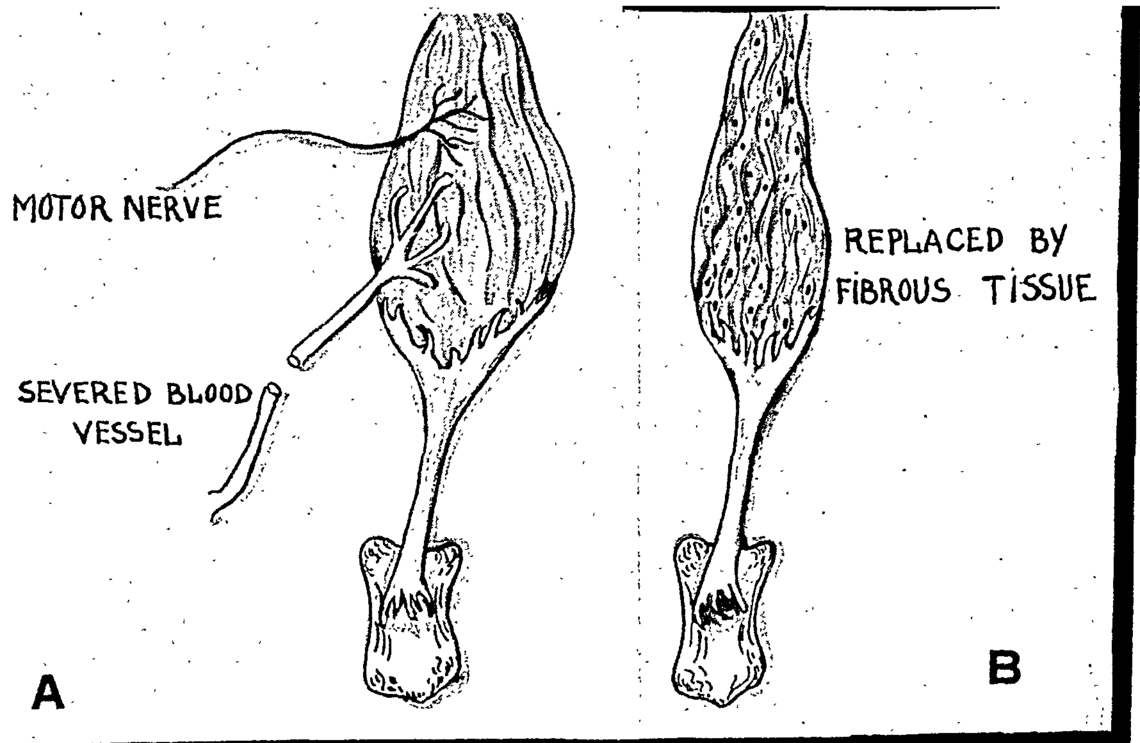


FIG. 146. A. Autogenous human skeletal muscle *in situ* with intact motor nerve but interrupted blood supply. B. Muscle cells die in a matter of hours and muscle is replaced by fibrous tissue.

affect the survival of complete muscle cell entities in the graft which has no circulation to remove them.

After section of either the muscular nerves or the motor nerve roots there is a perceptible reduction in the diameter of muscle fibers as early as one month, and the nuclei become more scattered (58). At the end of two months the decrease in diameter of muscle fibers is far advanced, and eventually only a string of darkly-staining rounded muscle nuclei remains. The small muscle fibers of the muscle spindles, however, seem peculiarly resistant to the process of degeneration atrophy.

Many attempts have been made to prevent the degeneration of muscle *in situ* with severed motor nerve by means of repeated electrical stimulation (38, 60). Electrical stimulation delays the loss in weight of paralyzed muscles for a short time, but after 4 weeks the treated muscles lose as much weight as the controls. Furthermore, the speed in recovery of function after reinnervation is not significantly altered.

After successful nerve suture, axons from the proximal end of the nerve reinnervate the muscle fibers. Some of the regenerating axons fail to reach the motor end-plates, and new motor end-plates are formed where these fibers contact the muscle fibers. After the regenerating axons reach the muscle fibers, atrophy no longer progresses and some degree of muscle function may be restored. Usually the bulk and strength of the reinnervated muscle are less than normal because some of the nerve fibers fail to establish contact with the muscle fibers. These remain atrophic and eventually degenerate.

Atrophy of disuse, as when a muscle group is inactivated by putting the arm in a plaster cast, *does not cause the severe degree of change as that which is seen when the motor nerve is cut*. Apparently, therefore, the various nerve impulses reaching the muscle through its intact nerve are important for the survival

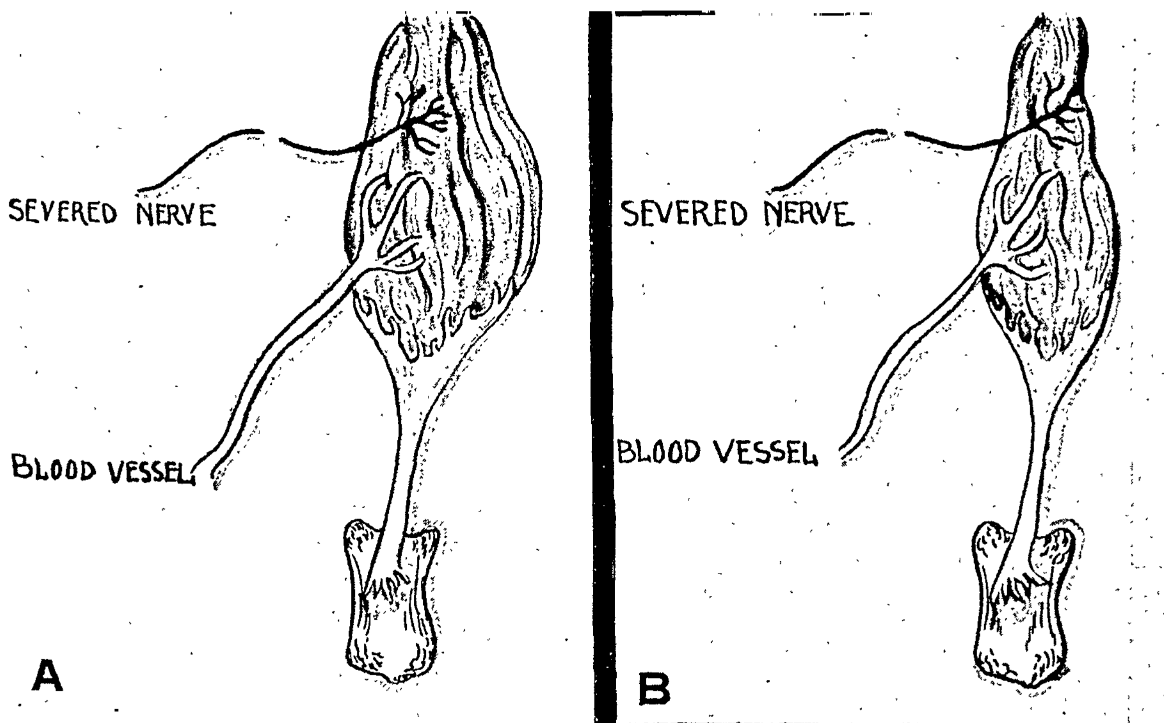


FIG. 147. A. Autogenous human muscle *in situ* with intact blood supply but severed motor nerve. B. The muscle cells very gradually undergo atrophy. This will stop if the muscle is reinnervated.

of the muscle cells in ways which are not clearly understood.

A common statement is that mammalian skeletal muscle has a negligible capacity to regenerate. As observed by Clark (61), regeneration can occur sufficiently to repair a very limited destruction of muscular tissue. This regenerative process involves two stages: 1) the removal of the necrotic muscle tissue by the action of macrophages, and 2) its replacement by the development of new fibers as outgrowths from the stumps of the old muscle fibers at the margin of the necrotic area. The preservation of the endomysial tubes of connective tissue which enclose the original fibers constitutes an important factor in the regeneration of necrosed muscular tissue. This is due to the fact that these tubes provide the guiding pathways for the newly-growing fibers. When muscle is undergoing severe muscle destruction, the replacement by fibrous tissue in the injured area causes obliteration of the endomysial framework and in this manner blocks growth of the old marginal muscle fibers from the stumps.

CLINICAL USE OF MUSCLE GRAFTS

As stated previously, the only use for free muscle grafts is to stop oozing from blood vessels in locations where ligation is not advisable. Thus, the late Dr. Harvey Cush-

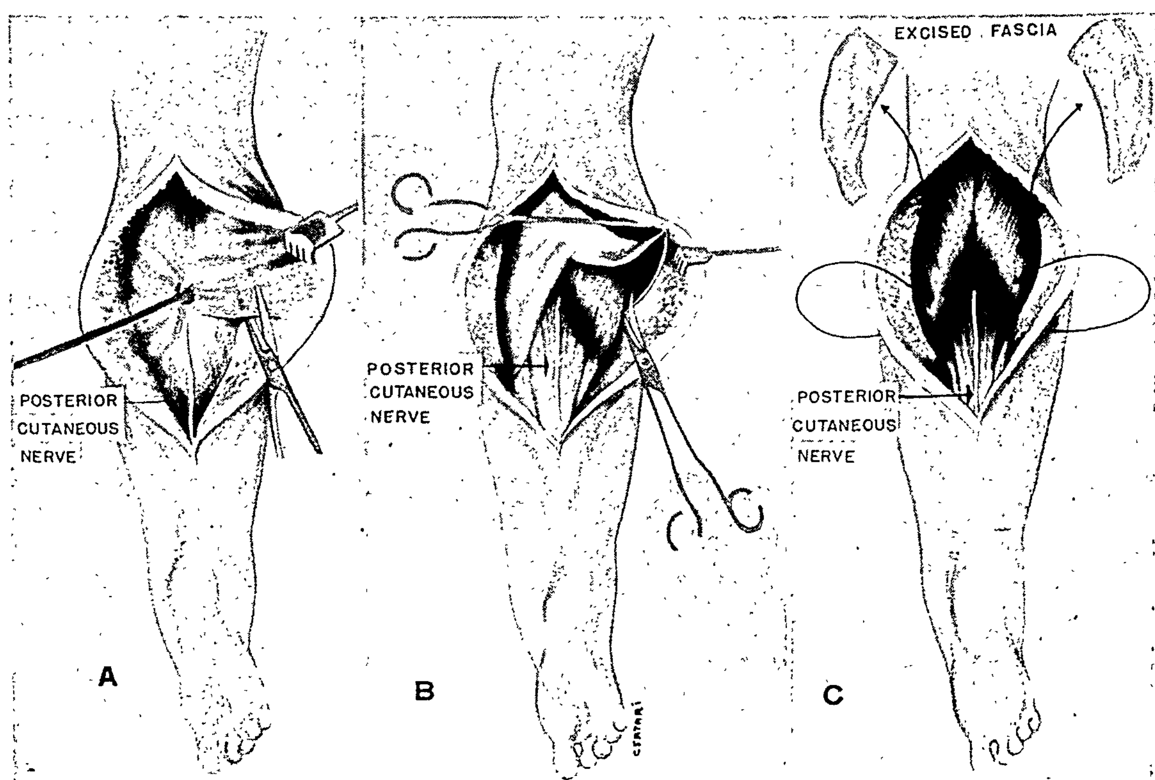


FIG. 148. Operation for Lymphedema of the Lower Extremity

In this procedure the thickened deep fascia is removed and the edematous subcutaneous tissue brought in direct contact with the muscle (gastrocnemius), which has a normal vascular and lymphatic supply. Interstitial fluid from the edematous subcutaneous tissues drains into the venules and lymphatics in this deep circulation.

A. Cutting deep fascia fibers away from subcutaneous fat.

B. Excising the deep fascia from the muscle.

C. Subcutaneous fat is laid directly over muscle after fascia has been removed. (Author's modified use of Kondoleon procedure for lymphedema of the leg.)

ing and other neurosurgeons applied small bits of free muscle over bleeding points on the surface of the brain.

Muscle flaps with intact nerve and blood supply have been advocated and successfully used for a rather wide variety of clinical purposes. Perhaps the most important is the shifting of a muscle with its tendon to provide motion or stability in a jointed bone to substitute for the absent or paralyzed muscle which would normally provide motion or stability. Such muscle shifts are frequently used by orthopedic surgeons and have reached a high level of intricate application by hand surgeons.

The masseter and temporalis muscles as flaps with intact nerve and blood supply are utilized to provide movement in soft parts, as in the skin and subcutaneous tissues of the face in facial paralysis. The gracilis muscle from the thigh is used by Kenneth Pickrell (62) to provide sphincter action around the

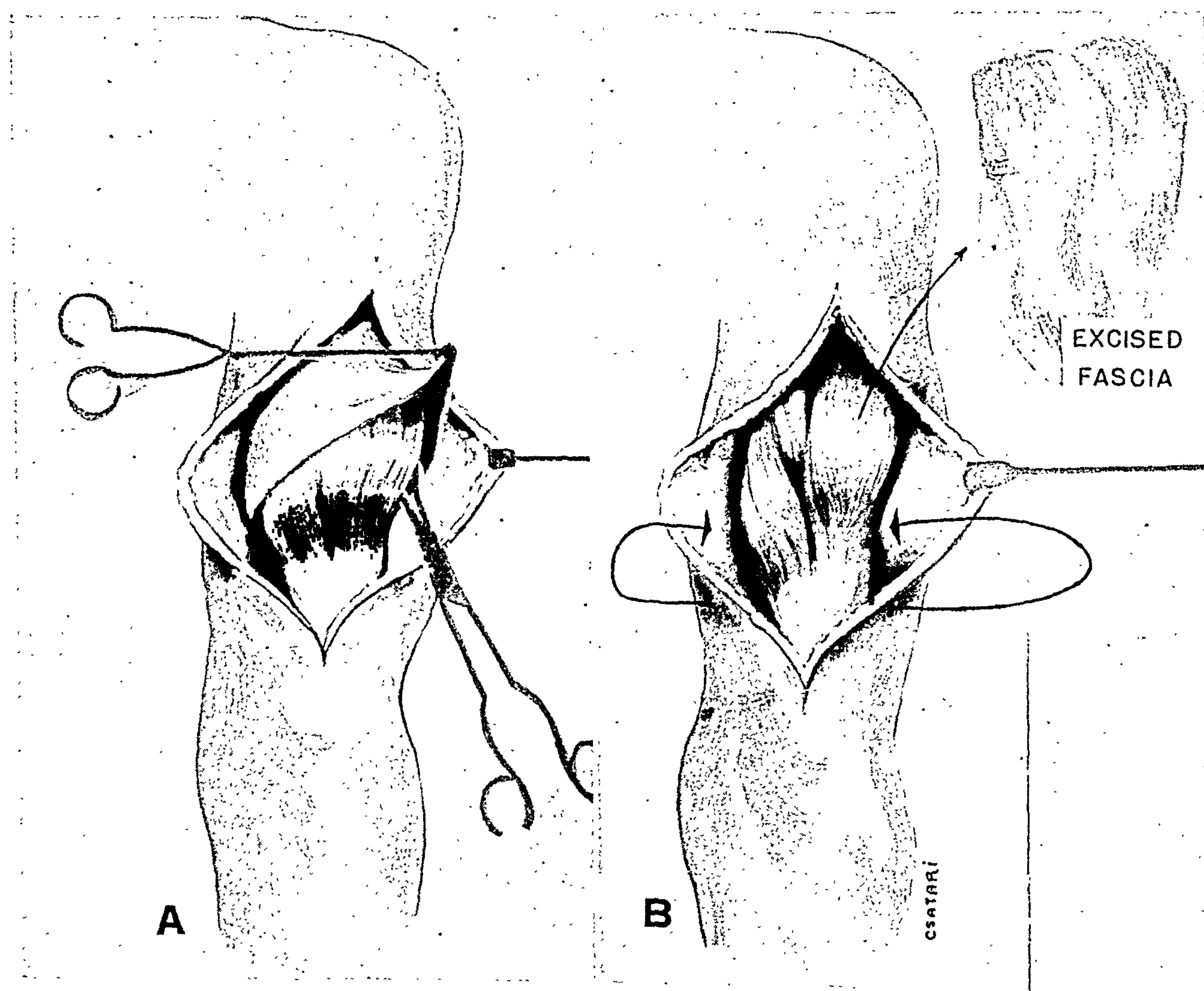


FIG. 149. Operation for Lymphedema of the Arm

In lymphedema of the arm following radical mastectomy, both the superficial and deep lymphatic vessels have been blocked operatively. When the thickened deep fascia over the triceps muscle is removed, interstitial fluid from the edematous subcutaneous tissues drains only into the venules.

A. Excising the deep fascia from the muscle.

B. Subcutaneous fat is laid directly over muscle after fascia has been removed. (Author's modified use of Kondoleon procedure for lymphedema of the arm.)

anus. Part of the superior rectus muscle of the eye may be shifted to substitute for the paralyzed levator muscle in ptosis of the upper eyelid. Ophthalmologists perform all manner of muscle shifts to correct strabismus of the eye and other positional abnormalities or deficiencies in synchronized movement which affect both the vision and appearance of the patient.

Muscle flaps have also been widely used to fill cavities in the lung and in bone with varying degrees of success. Muscle flaps have been brought into contact with the heart in coronary disease to provide a more adequate heart muscle circulation.

In a similar way latissimus dorsi muscle flaps have been transferred to the upper arm to provide better circulatory exchange in lymphedema of the upper extremity following radical breast amputation for cancer. The author prefers to remove the thickened and impervious deep fascia over the triceps in such patients, and bring the edematous subcutaneous tissue in direct contact with the vascular muscle. Sometimes the deep fascia over the biceps and in the forearm is also removed to provide a wider area of venous drainage. The larger proportion of interstitial fluid finds its way into venules rather than into lymphatic vessels under normal conditions, and in lymphedema

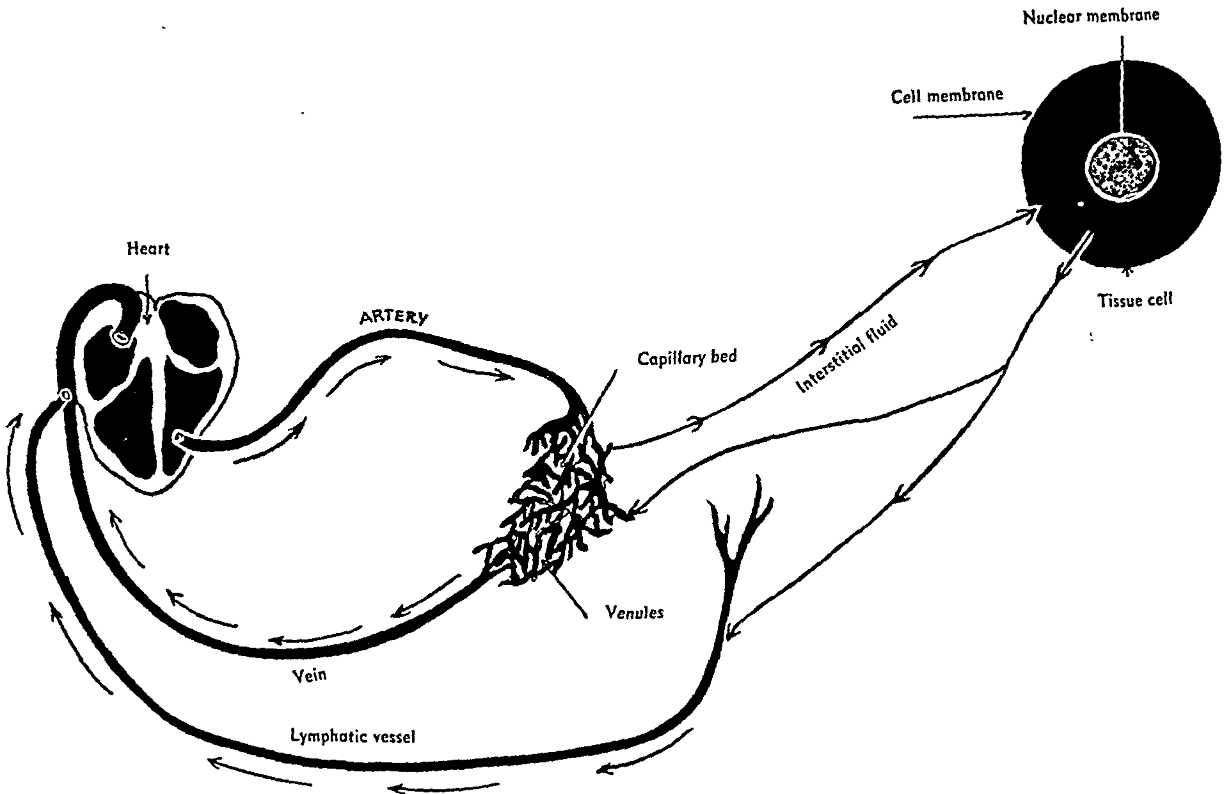


Fig. 150. Schematic drawing of the circulation from capillary bed to each tissue cell. Most of the interstitial fluid returns to the vascular system through the walls of the venules. A smaller amount returns through the lymphatic vessels. After radical mastectomy, all or most of the superficial and deep lymphatic vessels have been blocked. When edematous subcutaneous tissue is brought into contact with vascular triceps muscle, interstitial fluid must return into the closed vascular system almost entirely through the walls of the venules. In the operation for lymphedema of the lower extremities, interstitial fluid may return to the closed vascular system either through venules in the muscle or lymphatic vessels in the muscle, because the deep lymphatics have not been blocked.

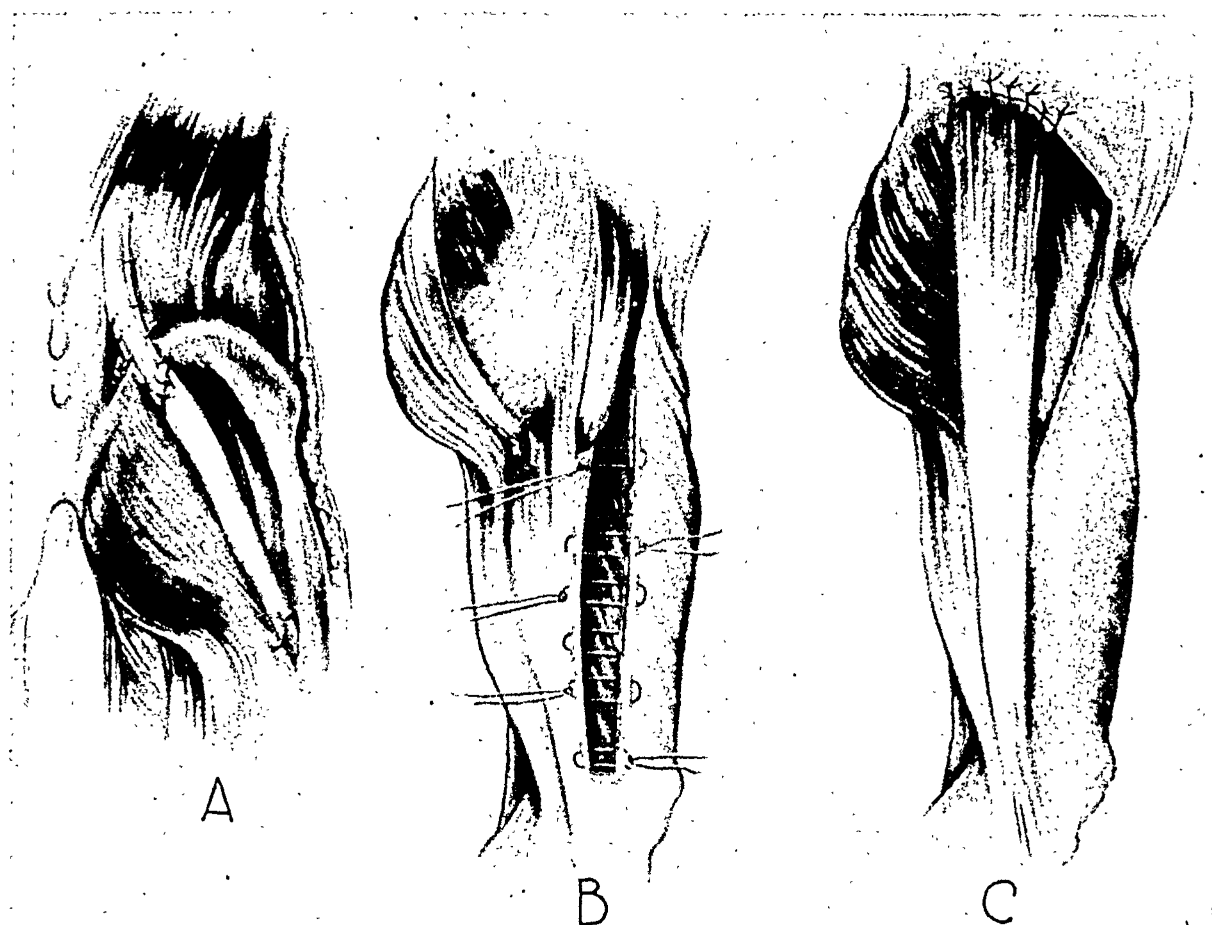


FIG. 151. Transference of erector spinal and tensor fascial femoris for paralysis of gluteus medius and maximus muscles (Ober).

A. Outer half of erector spinal group detached and sutured to fascia lata.

B. Long flap of fascia lata, 1½ inches wide, has been dissected free from thigh up to and including fibers of tensor fasciae femoris muscle. Flap passed from above backward, beneath muscles and periosteum 2 inches below base of trochanter and firmly sutured at entrance anteriorly and to gluteus maximus tendon posteriorly. Defects in fascia closed with mattress sutures (Ober).

C. Transference of tensor fasciae femoris for weakness of gluteus medius muscle (Legg). Origin of tensor fasciae femoris detached and transferred posteriorly.

From Plastic and Reconstructive Surgery, Earl C. Padgett and Kathryn L. Stephenson. Springfield, Ill.: Charles C Thomas, 1948.

of the arm after the thickened fascia has been removed, a large part of the interstitial fluid in the subcutaneous tissues probably goes directly into the muscle venules, since the lymphatics in the axilla have been largely blocked in the radical breast-amputation procedure.

The thickened deep fascia is also completely removed to provide better drainage for lymphedema of the leg, without removing any of the skin or subcutaneous tissues, as advocated in the Kondoleon[2] and other

[2] Deep fascia covering muscle has considerable regenerative powers, as was noted by Kondoleon. His most successful cases were those in which he removed about one-half of the deep fascia in lymphedema of the leg; he was fearful of removing more. In the author's modification of Kondoleon's basic conception all or practically all of the deep fascia is removed. The same principle has been applied for lymphedema of the arm following radical mastectomy. The physiological principles of the procedures, however, are those emphasized by Kondoleon.

operations. In this simple procedure of removing the thickened obstructive fascia, the edematous subcutaneous fat and covering skin are brought into direct contact with the vascular muscle, which serves to provide new drainage through its venules and lymphatics. Naturally the patients in early cases with minimum fibrosis obtain the most relief.

Some men have brought muscle flaps or free muscle grafts to recipient sites on the surface of the body. This is not sound physiologically, since muscle, like fascia and tendon, is normally covered and protected by skin, mucous membrane, and subcutaneous tissues. Muscle flaps or free grafts should be used therefore as buried grafts and not as surface grafts.

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operations. In this simple procedure of removing the thickened obstructive fascia, the edematous subcutaneous fat and covering skin are brought into direct contact with the vascular muscle, which serves to provide new drainage through its venules and lymphatics. Naturally the patients in early cases with minimum fibrosis obtain the most relief.

Some men have brought muscle flaps or free muscle grafts to recipient sites on the surface of the body. This is not sound physiologically, since muscle, like fascia and tendon, is normally covered and protected by skin, mucous membrane, and subcutaneous tissues. Muscle flaps or free grafts should be used therefore as buried grafts and not as surface grafts.

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PART VI

Theories of Cell Regeneration

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CHAPTER 30

Cell Survival Theory Versus Replacement Theory

In the previous chapters we have seen that free grafts of cartilage, bone, fascia, and tendon in favorable transplantation sites appear like the same identical tissue when they are exposed or removed and examined grossly and microscopically. The question arises as to whether the cells and matrix of the grafts survive transplantation as such, or, alternately, are replaced by infiltrating host fibroblasts as the same kind of tissue.

Many investigators believe that certain cells persist in the adult organism *with the potency of undifferentiated mesenchymal cells.* Under the influence of certain stimuli these undifferentiated cells may undergo progressive development and furnish new cell types (1).

There is some evidence, however, *that any ordinary fibroblast may revert to an undifferentiated connective-tissue cell* if the need arises; this has been demonstrated in tissue culture growth of adult human fibroblasts. Those who oppose this theory point out that adult tissues growing in tissue culture media often behave quite differently from tissues in the body. For instance, epidermal cells in culture media may grow indefinitely. In the body, however, they will respond to growth stimulators and cover a raw wound where epidermis has been destroyed, but when this has been accomplished the cells stop growing due to some growth inhibitor substance or other inhibitory factors which are absent in tissue culture media.

The majority opinion is that fibroblasts in the connective tissues of the body are differentiated cells which do not give rise to other types of connective tissue. When new types of tissue are formed, such as bone in or about cartilage grafts transplanted in fat, the bone formation occurs through the activity of undifferentiated mesenchymal cells. The undifferentiated mesenchymal cells[1] are often smaller than fibroblasts but have the same general appearance; in the loose connective tissue they are usually arranged along the blood vessels, particularly along the capillaries. Cowdry (2) very aptly remarks that the hang-over primitive mesenchymatous cells are so convenient to think of and so hard to find. Certainly great vagueness is manifest in statements as to what tissues the mesenchymatous cells accurately replace, and why they replace the viable cells in free grafts when there is no apparent need for this replacement. In effect, positive evidence supporting the replacement of the cells in many free grafts that have survived transplantation is obscure.

[1] Often called primitive mesenchymal cells.

and absorbed by the host tissues. Those areas where absorption has occurred, however, are occupied by host fibrous tissue or new host bone formation rather than by new cartilage.

One can therefore probably rule out the host tissue replacement theory as a valid explanation of action in the great majority of autogenous cartilage grafts in humans.

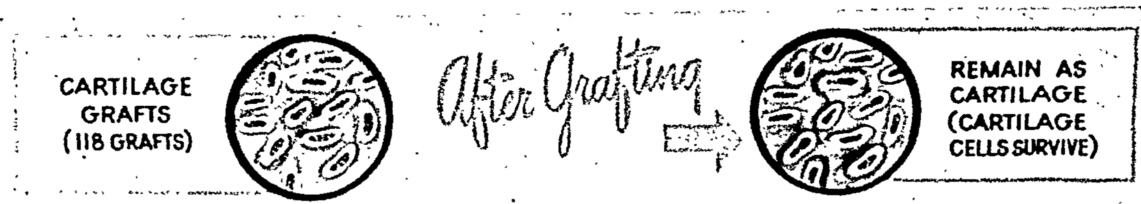


FIG. 152. Autogenous cartilage grafts[2] of all varieties, either with or without perichondrium, remain as cartilage. The chondrocytes survive as living cells associated with their specific matrix structure (hyaline, elastic and probably fibrocartilage).

Cartilage grafts retain their matrix and viable chondrocytes regardless of whether they are in contact with cartilage or with fat or muscle.

Bone Grafts

In a consideration of bone grafts we must study the behavior of the common bone grafts, which are iliac, rib and tibial bone in one group and others, which are largely membranous, such as septal, nasal and turbinate bone grafts in a separate category, since the two groups behave differently.

In rib, tibial and iliac bone grafts functional use is essential for retention of the calcified matrix of the transplants. Thus, these bone grafts must be transplanted in contact with living host bone. When the grafts are transplanted in soft tissue sites, the calcified matrix is slowly absorbed and replaced by fibrous tissue. The bone cells remain as living cells, however, until their calcified matrix has been removed. Then they disappear among the multitude of host fibroblasts. Their subsequent fate is not known (3, 4, 5).

Most of the cells in cancellous bone grafts appear to survive *en masse* when the graft is in contact with living bone. Healing occurs by callus formation as in healing fractures. In dense cortical bone grafts where the cells die because of nutritional difficulties, the dead graft structure may be replaced by ingrowth of cells from the host bone, and/or its periosteum. Possibly osteogenetic cells from the surrounding connective tissue participate in this process of creeping substitution. *This is the one known or accepted example where host tissue infiltrates an autogenous graft and actually replaces the graft structure, so that it resembles the original tissue.*

When septal, nasal and turbinate bone grafts are transplanted in soft tissue sites, the osteocytes remain as living cells and the calcified matrix of the graft is retained up to five years after transfer, which is the longest time that such grafts have been followed by the author. The blood vessels in the grafts with their endothelial cell lining also survive, and there is no positive evidence that the bone cells have been replaced by infiltrating host cells. This latter observation is based on a study of grafts removed at intervals of a few days after transfer and on serial examination for longer periods of time.

Fascia Grafts

The survival of the fibroblast cells in autogenous fascia grafts is admittedly not as easy to demonstrate in a definite or positive manner as that of the chondrocytes in cartilage grafts. While it is possible to be reasonably sure that there is no mass invasion of

[2] *Note:* Since these drawings were made, additional transplants of all tissues excepting nerve have been removed and examined microscopically.

Host Tissue Replacement Theory

The great activity of the fibroblast in wound healing and the fact that bone grafts in contact with host bone can be replaced by new bone formation (creeping substitution) were noted by many early investigators. It was quite natural, therefore, that these men should believe as a generalization that either free grafts were absorbed and replaced by connective tissue, or, if the graft structure remained as in autogenous cartilage, fascia, tendon and bone grafts (in contact with bone) *host tissue cells had gradually infiltrated the grafts and replaced the original cell and intercellular substance* in such a clever way that the "counterfeit model" was an exact duplicate of the original graft.

The theory of gradual replacement of free autogenous grafts by infiltrating host cells in such a manner that bone is replaced as bone, cartilage as cartilage, fascia as fascia, and tendon as tendon, has dominated the thinking of past investigators and still influences the reasoning of present-day experimenters and clinicians. It is possible that *the replacement theory is without foundation in fact regarding most autogenous human tissue grafts and is known to apply only to some bone grafts and to nerve grafts to a limited extent.*

After careful study of the behavior of ten commonly used free tissue grafts in humans the author concluded that the great majority of these grafts probably *survive* and are not replaced by host tissue cells. This general tendency for the cells in free grafts to remain viable after transplantation is described in this chapter as *the cell survival theory.*

BEHAVIOR OF FREE HUMAN AUTOGRAFTS OF FIVE BASIC TISSUES

Cartilage Grafts

On the basis of abundant factual evidence one can state that autogenous cartilage grafts are not absorbed and replaced as cartilage in the human.

It is quite easy to study the behavior of cartilage grafts because they contain only one living entity, the chondrocytes, which are surrounded by a relatively large amount of durable non-living matrix. Any infiltrating host tissues such as blood vessels or fibroblasts can be readily seen on microscopic examination. If an autogenous cartilage graft buried for 20 years is sectioned in the fresh unfixed state, the observer may note that the cartilage cells are normal in appearance and look exactly like the chondrocytes in fresh control sections of cartilage. The cells in the graft and in the control cartilage both take supravital dye like living cells (only the cytoplasm takes the stain). As the sections dry, the cells lose their content of moisture, retract from the walls of their lacunar spaces, and resemble the dead cells in fixed sections of cartilage. If the observer carefully studies the fresh section of the cartilage graft and can see no evidence of host tissue infiltration in the graft structure, then it can be assumed that the cartilage cells in the graft *have survived.*

I have examined numerous fresh sections of autogenous cartilage grafts in the fresh state, as described above, and have found viable cartilage cells in autogenous rib, septal and ear cartilage grafts buried in humans for various periods of time. The oldest graft was rib cartilage buried under the nasal skin and removed 27 years after transplantation.

All autogenous cartilage grafts buried in the human do not behave in exactly the same way. In occasional grafts some invasion of the graft by host fibroblasts accompanied by blood vessels may be seen, but new cartilage formation in or about a cartilage graft has never been observed. Curiously, new bone formation may occur in or about human cartilage grafts.

Thus, in autogenous cartilage grafts there is very definite evidence that the chondrocytes tend to survive as such and, in general, retain all or most of their intercellular matrix. Portions of the graft may be invaded

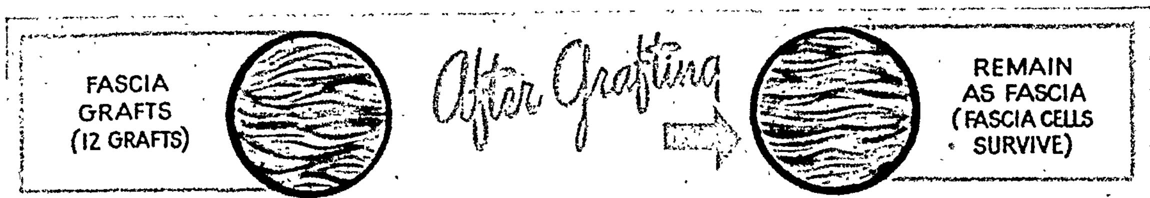


FIG. 154. Autogenous fascia grafts in contact with fascia or in fat retain their specific structure and the fascial fibroblasts remain viable.

through the blood stream and pass through the thin capillary walls into the intercellular substance of the graft by diapedesis. These cells which are present in the dilated capillaries are often seen in the act of passing through the capillary walls. The cells are identifiable as polymorphonuclears, eosinophiles, plasma cells, and lymphocytes. As might be supposed, fibroblasts as such are not observed in the exudate since these do not appear to travel through the blood vessels. The graft fibroblasts in fascia grafts buried for 3 days have dark staining nuclei and are more numerous than those seen in control fascia. Thus it appears that the graft fibroblasts undergo early proliferation, so that their numbers have been increased. This increased number of fibroblasts seems to persist in transplanted fascia after the cellular exudate has disappeared and the graft structure appears normal in all other respects. Grafts buried for 1½ years still show a larger number of fibroblasts than control fascia, and the nuclei of these cells stain rather dark. In all other respects the autogenous fascia grafts appear entirely normal 1½ years after transplantation in humans.

Tendon Grafts

Thin and moderately thick tendons behave like fascia transplants in humans. The evidence for survival of the tendon cells and fibroblasts in the stroma of free tendon grafts is about the same as that for the survival of the fascial fibroblast. The arguments against the general tendency for survival of the cells are negative but still should be given due consideration.

It would be helpful for some investigator to destroy the cells in a fresh autogenous tendon by desiccation and then bury segments in separate transplantation sites. Removal and examination of these grafts at intervals up to 1½ years might enable one to determine the ability of the host fibroblast to infiltrate the graft structure and replace the tendon and stromal cells, so that the tissue did or did not resemble tendon.

Serial sections of fresh autogenous tendon grafts buried with paratenon for a few days up to eight months show the same general picture as fascia grafts. Early circulation is established (in about three days) by anastomosis btween host and paratenon blood vessels. The tendon cells, stromal fibroblasts, and endothelial cells in the graft blood vessels all appear to survive associated with the collagenous fiber matrix. Dead or dying tendon, stromal or endothelial cells have never been observed by the author.

Muscle Grafts

The cells in free muscle grafts die in a matter of a few hours after transplantation

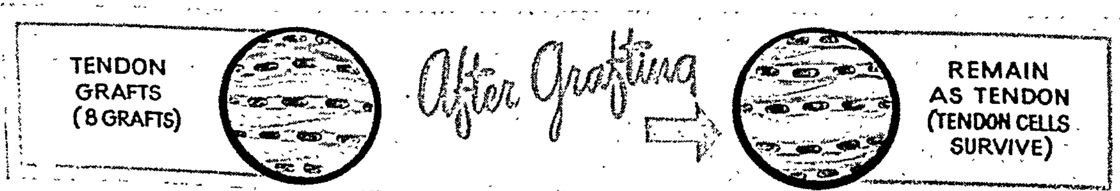


FIG. 155. Autogenous tendon grafts in contact with tendon or in fat retain their specific structure and the tendon cells remain viable.

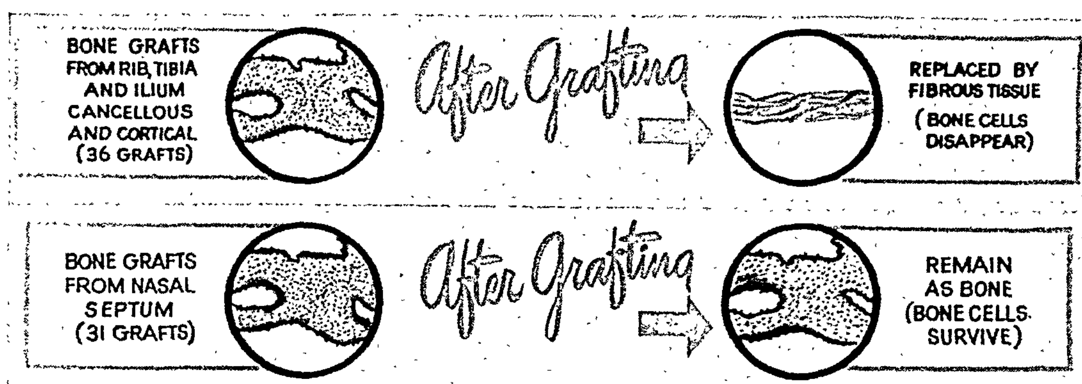


FIG. 153. Autogenous bone grafts from the rib, tibia, or ilium, either cancellous or cortical, are replaced by fibrous tissue after transplantation in fat. When transplanted in contact with bone, either they partly retain their structure (cancellous), or they are replaced as bone (cortical).[3]

Autogenous bone grafts from the nasal septum, turbinate, or nasal bones, without periosteum, remain as bone after transplantation in fat.[4]

host fibroblasts in fascia grafts examined from one day up to one month at intervals of a few days each, still it is not easy to rule out the penetration of the graft by host capillaries accompanied by supporting host fibroblasts or undifferentiated mesenchymal cells. In my experience fascia grafts in humans removed at intervals of 1 to 12 days do not always show the same microscopic picture. Some fascia grafts in humans show very little infiltration of the graft structure by host tissue cells of the exudate type, whereas others have dense collections of polymorphonuclears, lymphocytes, eosinophiles, and the like within the graft structure. However, eventually these exudate cells disappear, and the graft appears like normal control fascia grossly and microscopically.

Certainly the blood vessels in the graft and their endothelial cell lining do survive, and early circulation is established through end-to-end anastomosis between host and graft blood vessels. Some penetrating growth by host capillaries does occur, however, and one must admit that the fibroblasts accompanying these vessels could, in theory, replace fibroblast cells in the graft.

The evidence against the host tissue replacement is at least stronger than the evidence for it, since no degenerating or dead fascia graft fibroblasts have been observed. A cellular exudate is noted in numerous areas within the graft substance after the blood circulation has been established, but this results from various cells which travel

[3] *Note:* This replacement phenomenon is known as "creeping substitution," and it is the one known or accepted example in which host tissues rather accurately remove and replace the graft structure and cell population. The behavior of the grafts is not dependent on the presence or absence of periosteum.

[4] *Note:* Complete autogenous finger and toe bones (phalanges) with ligaments, joint cartilage and nails remain as bone after transplantation in abdominal fat (twenty-two months). The ligaments, joint capsules, cartilages and nail-beds, from a gross standpoint, appear normal and the individual bones are freely movable. These experimental findings (unpublished work by the author) suggest many clinical applications for patients with amputated fingers, transference of bone with nail-bed from supernumerary fingers and the like.

Three finger bones were transplanted in two patients, two toe bones in one patient, and a complete metacarpal bone in one patient. The finger bones were transplanted with periosteum and the metacarpal, without periosteum. All have retained their calcified structure as determined by palpation beneath the skin and by exposure through a skin incision in one patient (finger bones buried for nineteen months).

the other commonly-used autogenous tissue grafts which will be described with organs, blood vessels, fetal tissues etc. in Volume II. These tissues are: surface skin, surface mucous membrane, buried skin, fat, and peripheral nerve tissue.

Surface Skin Grafts

The epidermal portions of split or full thickness skin grafts are certainly not replaced by infiltrating host epidermal cells or host fibroblasts. This is easily demonstrated by the survival of hairs and glands in the dermis and the fact that pigmented nevi and other elevated growths remain after transplantation. Incidentally the fact that angiomas remain in skin grafts after free transplantation strongly indicates that the vascular system survives as such.

Skin grafts transplanted in contact with unlike tissues such as ciliated mucous membrane and as isolated patches over bone retain their gross and histologic structure as skin. In these locations there are no host cells which can infiltrate the skin graft and replace the hair follicles, glands, and epidermal covering.

The evidence for survival of the connective-tissue portion of dermis in skin grafts as such is about the same as that for the survival of fascia and tendon grafts after transplantation. No investigator has observed host fibroblasts in the process of replacing the fibroblasts in the transplanted dermis but the old idea occasionally appears in the literature. Theoretically it is possible but it has never been noted and reported.

The positive evidence from microscopic examination of skin grafts at intervals from 1 to 14 days indicates rather strongly that the fibroblast cells in the dermis survive and are not replaced by host fibroblasts. A skin graft removed and examined 40 years (6) after transfer appeared exactly like normal control skin, and special staining demonstrated that elastic fibers were present in the dermis.

Surface Mucous Membrane Grafts

Mucous membrane grafts after free transplantation behave in the same manner as skin grafts.

Buried Skin Grafts

When full thickness skin or the dermis of skin is completely buried, the transplantation site is not a normal or favorable one. This is allowed for in the cell survival theory.

The epidermis, when this is present, the hairs, and the glands are all normally on the body surface or have a connection with the outside. When these epidermal structures are buried and not allowed to breathe, they usually degenerate and disappear. The fibroblast cells in the dermis and their collagenous and elastic fibers are normally buried and hence the dermis remains as such in buried skin and dermal grafts.



FIG. 157. Autogenous surface skin grafts remain as skin regardless of whether they are in contact with skin or mucous membrane, or are isolated islands over bone. The cells in the epidermis, hair follicles, glands, and special-sense organs all appear to survive as such. The fibroblast cells in the dermis also appear to survive associated with their normal intercellular substances.

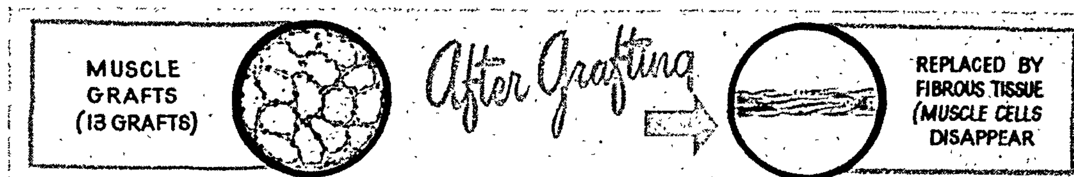


FIG. 156. Autogenous skeletal muscle grafts are replaced by fibrous tissue regardless of whether they are transplanted in contact with muscle or in fat. The muscle cells or fibers degenerate and disappear.

due to severance of their blood supply. The muscle cells die regardless of whether the free muscle segment is transplanted in contact with muscle or in fat. In either transplantation site the graft is replaced by fibrous tissue and *never as muscle.* Most of the fibrous tissue replacement of the muscle cells occurs through proliferation of fibroblasts in the stroma of the graft itself. These fibroblasts in the free muscle transplant and their supplying blood vessels appear to survive.

Summary Comment

A careful study of the behavior of 4 of the 5 free tissue grafts described in this volume indicates that *the cells show a tenacious ability to survive as living entities.* In grafts of cartilage, cancellous bone in contact with bone, septal, nasal, and turbinate bones, fascia, and tendon, the cells tend to retain the specific structure of their matrix. Thus, cartilage remains as cartilage, bone as bone, fascia as fascia, and tendon as tendon. There is no positive evidence in any of these grafts that the parenchymal cells are replaced by infiltrating host cells which take over and maintain the graft structure or absorb and replace the original matrix (excepting portions of cancellous bone).

The muscle cells in free muscle grafts die in all transplants but *the graft structure is replaced as fibrous tissue and not as muscle.*

Only in dense cortical bone grafts in contact with bone (and probably in portions of cancellous bone grafts) may the graft structure and cell population be replaced by the host tissue in kind. This is to say that the dead cells and graft structure are gradually absorbed and replaced through the activity of infiltrating host cells, so that eventually the original graft is replaced by a counterfeit model, which is a duplicate of the original transplant. This process of creeping substitution is generally accepted insofar as it applies to bone grafts with dead cells in contact with living host bone.

CELL SURVIVAL THEORY

The tenacious and usually successful effort of the cells in most free autogenous human grafts to survive as living entities is an impressive fact. One may therefore postulate a "Cell Survival Theory" in opposition to the "Host Tissue Replacement Theory." This cell survival theory applies to all of the human tissues considered in this volume with the exception of dense cortical bone, and also to other commonly-used tissue grafts, which will be described in Volume II and are only briefly mentioned in this chapter.

The cell survival theory is stated as follows: *"In humans the cells in free autogenous grafts tend to survive and retain their normal tissue structure when transplanted as complete cell entities in favorable transplantation sites." When the cells in free grafts fail to survive, the graft is replaced by connective tissue but this replacement is not a duplicate of the original graft.*

Application to Autografts of Other Basic Tissues

Let us now consider in a general way how the cell survival theory applies to some of

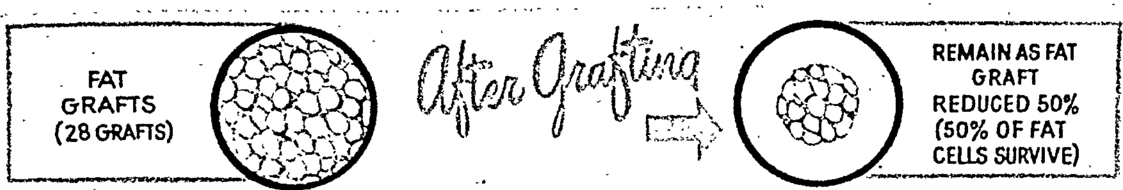


Fig. 159. Autogenous fat grafts in fat or within the rectus sheath remain as fat but the number of cellular constituents in the graft are reduced, and hence the bulk of the graft is usually less. About 50 per cent of the fat cells rupture and die but the cells which do not rupture tend to survive, and these collectively form the fat graft that remains. Abdominal fat grafts will increase in size if the patient lays on additional abdominal fat. This, however, is probably due to an increase of intracellular fat in the surviving fat cells and not to an increase in the number of cells.



Fig. 160. When an autogenous peripheral nerve is transplanted in contact with unlike tissue such as fat, the axons, myelin sheath and Schwann cells disappear. The fibroblast cells in the endoneurium, perineurium, and epineurium survive associated with their intercellular material.

When an autogenous nerve graft is transplanted in contact with nerve, the axons, myelin sheath and Schwann cells also disappear. New axons, however, grow down through unobstructed channels in the graft from the proximal host nerve but the fibroblast cells in the neurium of the graft survive. The new axons develop myelin sheaths though an unknown agency and Schwann cells are said to grow down from the proximal nerve. *Possibly some or all of the original Schwann cells in the graft survive and participate in the repair.*

Thus when nerve is in contact with nerve there is replacement of the axons, which are cytoplasmic extensions of the host nerve cells and not complete cell entities. Many of the Schwann cells in the nerve graft may survive and produce the new myelin sheath substance.

definitely survive and undergo early proliferation, which indicates that they are alive. The Schwann cells may also survive, since no observer has reported the presence of a dying or dead Schwann cell in a free nerve graft.

Resumé

From the foregoing it is seen that possibly all of the commonly used free autogenous human tissue grafts comply in behavior with the cell survival theory excepting dead bone in contact with living host bone. The question of cell survival in a free nerve graft involves only the survival of the Schwann cells in the graft, and no one knows that surviving Schwann cells in the graft do not participate in lining new axons which penetrate the graft.

The available evidence indicates that free grafts of cartilage, most bone grafts, tendon, fascia, muscle, surface skin, surface mucous membrane, buried skin, and fat may all conform to the cell survival theory and *not to the host tissue replacement theory.*

SURVIVAL OF BLOOD VESSELS IN FREE GRAFTS

The blood vessels with their lining endothelial cells tend to survive in successfully transplanted autogenous human tissue grafts. Indeed, one may turn this statement



FIG. 158. Autogenous skin grafts buried in fat appear to lose all of the epidermal cells and epidermal derivatives which normally have contact with the outside surface. The cells in the epidermis, glands, and hair follicles degenerate, and these structures tend to disappear in humans. The fibroblast cells in the dermis survive and retain their normal matrix constituents.

Fat Grafts

The old belief (still accepted by some) that all of the fat cells in a free autogenous human fat graft die after transplantation and that the graft fat cells are partly replaced by infiltrating host cells which take on fat and become fat cells *is not supported by factual evidence* in animals (7) or in humans (8).

Actually, the more hardy or more advantageously-located fat cells in the graft appear to survive, and these cells collectively represent the fatty tissue which remains for a year or more after transplantation.

The fibroblast cells in the stroma between fat cells also survive in free fat transplants, and the vascular system in fat grafts tends to survive as such, with its endothelial cell lining. Early circulation is established through end-to-end anastomosis between graft and host blood vessels. This is the usual occurrence in all free grafts which normally have a blood vessel supply although some capillary ingrowth takes place. Whether this penetrating capillary ingrowth later anastomoses with the established graft vascular system, forms a separate disconnected circulation or eventually disappears, is not known at this time.

One speculates as to whether the surviving fat cells in fat grafts are the original cells or their descendants. According to Cowdry (2), the assumption that fat cells are short-lived is unwarranted. Dividing fat cells are scarcely ever seen and the same can be said of dying ones. Thus the normal life span of the fat cell, like that of most tissue cells, is not known.

Peripheral Nerve Grafts

In free nerve grafts in contact with nerve, the axon or process which has been severed from the nerve cell proper always degenerates and dies together with its non-living myelin sheath. The degeneration of the axon in a nerve graft conforms to the cell survival theory since it is not a complete cell entity. No one knows the fate of the Schwann cells in free nerve grafts. I have observed that they appear to survive the initial shock of transplantation but that they later disappear among the large numbers of proliferating endoneurial fibroblasts. These endoneurial fibroblast cells not only survive but also proliferate to such an extent that they often block the pathways through which axons from the proximal nerve attempt to grow.

Under favorable conditions axons in the proximal end of the host nerve grow down through unobstructed channels in the graft, and these axons are said to be accompanied by host Schwann cells, which probably elaborate a new myelin sheath.[5] This, if true, is a form of surface replacement like the growth of skin epidermis across a raw surface. It is interesting to note, however, that the fibroblast cells in the neurium very

[5] There is disagreement among various authorities regarding the origin of the myelin sheath.

The blood vessels in the dermis of skin grafts and in buried grafts such as fat (8), bone, tendon, and other vascular tissues are at first filled with granular debris or in some grafts, (fat) with coagulated blood. Very often the smaller vessels appear empty in grafts buried for one and two days. During this interval, however, the endothelial cells lining the blood vessels appear viable in the stained sections. Usually by the third day circulation has been established in the smaller vessels which are not occluded by coagulated blood, through anastomosis between the host and graft blood vessels although the exact interval varies in different grafts. In stained sections early circulating blood is determined by the presence of normal red blood cells in large numbers and also by numerous white blood cells, which were absent in grafts buried for one and two days.

In the author's experimental study (8) of the developing circulation in autogenous human fat grafts some of the larger vessels became completely occluded by coagulated blood. Later (in about 8 to 10 days) some of these vessels became recanalized and circulation was reestablished.

A study of the developing circulation in the dermis of surface skin grafts, buried dermal grafts, and that of fascia and tendon grafts, removed at 24-hour intervals from 1 to 6 days, was as follows: In 24 to 48 hours the graft blood vessels appeared empty, with only a few clumped red blood cells within the vessels. White blood cells which had been trapped in the graft vessels at the time of transfer survived and migrated by diapedesis through the blood vessel walls into the interstitial substance of the graft. In about three days following transfer (and sometimes not until the fourth or fifth day) the graft blood vessels were distended with blood, and additional white blood cells infiltrated through the blood vessel wall into the graft structure, where they sometimes formed concentrated masses. The inference is that in about three days anastomosis occurred between host and graft blood vessels.

Additional evidence that this anastomosis actually does occur has been produced by transplanting full thickness skin grafts containing capillary nevi within the dermis of the graft. The red color in the skin disappears when the graft is detached and applied to a recipient site as a free autograft. In about 3 days the capillary nevi reappear, indicating that host blood vessels have anastomosed with the vascular system of the graft. Such transfers have been accomplished in 4 patients and the capillary nevi have remained in all instances (the oldest case having been observed for 6 years). Figure 163.

Conway (9) and his associates, using the transparent chamber technique in mice, observed what was interpreted as a capillary penetration of their grafts and this occurred later than in a period of 3 days following transfer.

Taylor and Lehrfeld (10), however, working with autogenous and homogenous skin grafts in rats, concluded that circulating blood in both graft types was established in about 3 days following transfer and they believed that this occurred through anastomosis between host and graft blood vessels.

With the transparent chamber technique it may be difficult to exclude the possibility that what appears to be capillary ingrowth is actually established graft capillaries which are receiving blood through anastomosis with host capillaries. Dr. Conway does not believe that his observations excluded the probability that end-to-end anastomosis between host and graft blood vessels does occur (11).

A great deal of earlier experimental work has been done to determine just how the new blood vessel supply develops in free surface skin grafts. John Staige Davis (12), for instance, stated that free skin grafts are at first nourished by serum from the raw

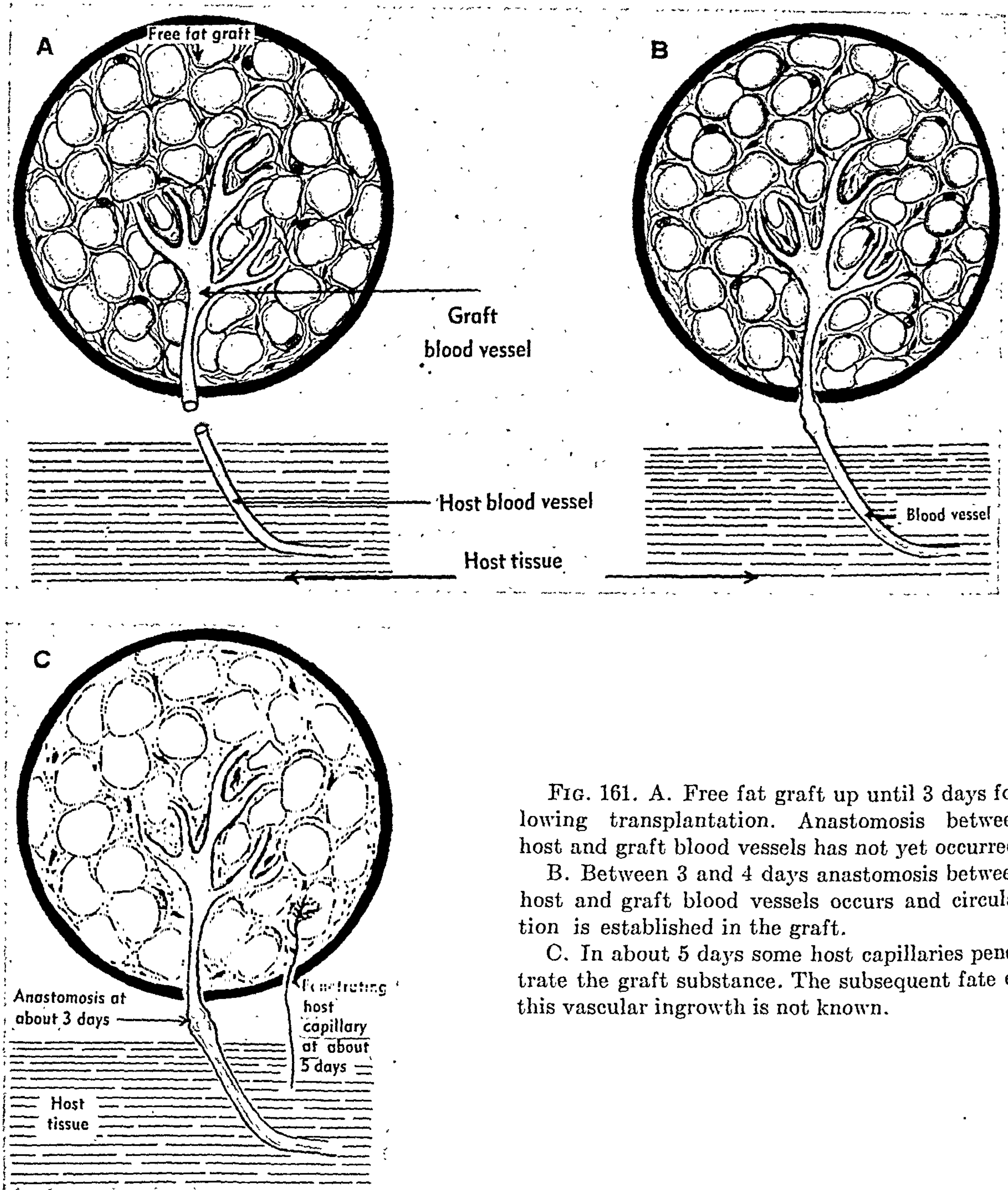


FIG. 161. A. Free fat graft up until 3 days following transplantation. Anastomosis between host and graft blood vessels has not yet occurred.

B. Between 3 and 4 days anastomosis between host and graft blood vessels occurs and circulation is established in the graft.

C. In about 5 days some host capillaries penetrate the graft substance. The subsequent fate of this vascular ingrowth is not known.

around and say that the survival of the blood vessels and the establishment of early blood circulation must occur (in about three days) or the cells in the graft will die from accumulation of waste products, and lack of oxygen and other necessary substances.

The above statement, of course, applies only to tissues which normally have a blood vessel system of circulation. Cornea, cartilage, lens, and the epidermis of skin do not have a vascular system, and they retain their normal mechanism of exchange by fluid permeation after free transfer. In free skin grafts dilated capillaries extend up to the basement membrane, but they never penetrate the epidermis, which retains its primitive method of circulation by tissue fluid infiltration.

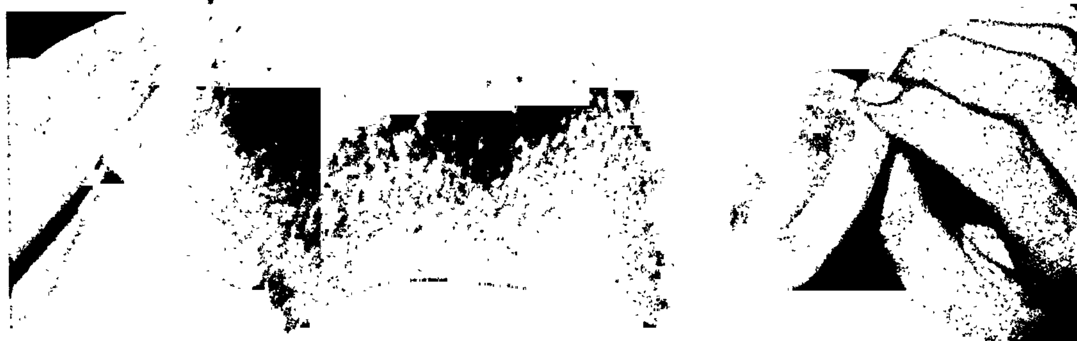


FIG. 163. Photograph of a patient who had two full thickness skin grafts from eyelids, with capillary nevi, transplanted to recipient sites behind the ears.

Note that the capillary plexus in both skin grafts survived transplantation and still serves as the vascular system of the grafts one and a half years after transplantation.[6]

Peer: Plast. & Reconstr. Surg. 5: 217, 1950.

surface on which they rest. This serum penetrates the old blood vessels and connective tissue of the graft. After about 22 hours the earliest blood circulation occurs through an anastomosis of capillaries of about the same caliber. Later there is an upward growth of capillaries from the host tissues inside of old vessels in the graft, and finally a penetrating invasion of capillaries occurs from the host into the tissues of the graft. On the basis of fixed and stained sections, Davis believed that this later penetrating capillary growth gives rise to the permanent circulation.

The observations made by Taylor and Lehrfeld on the developing circulation in skin autografts through direct microscopic examination of transplants *in situ* very closely resemble our own observations of fixed and stained sections of skin autografts in humans (6). Indeed, the findings and conclusions made by two different groups using different methods of observation in different species appear to be in agreement. Furthermore, the method and pattern of the developing circulation in skin autografts appears to be the same as that noted in other vascular autografts such as buried septal bone (4), fat (8), tendon, fascia,

[6] *Note:* The vascular system in all free autografts appears to survive transplantation, and the grafts receive early circulating blood through direct anastomosis between the graft and host blood vessels (surface skin, buried skin, fat, nerve, bone, tendon, and fascia grafts, etc.).

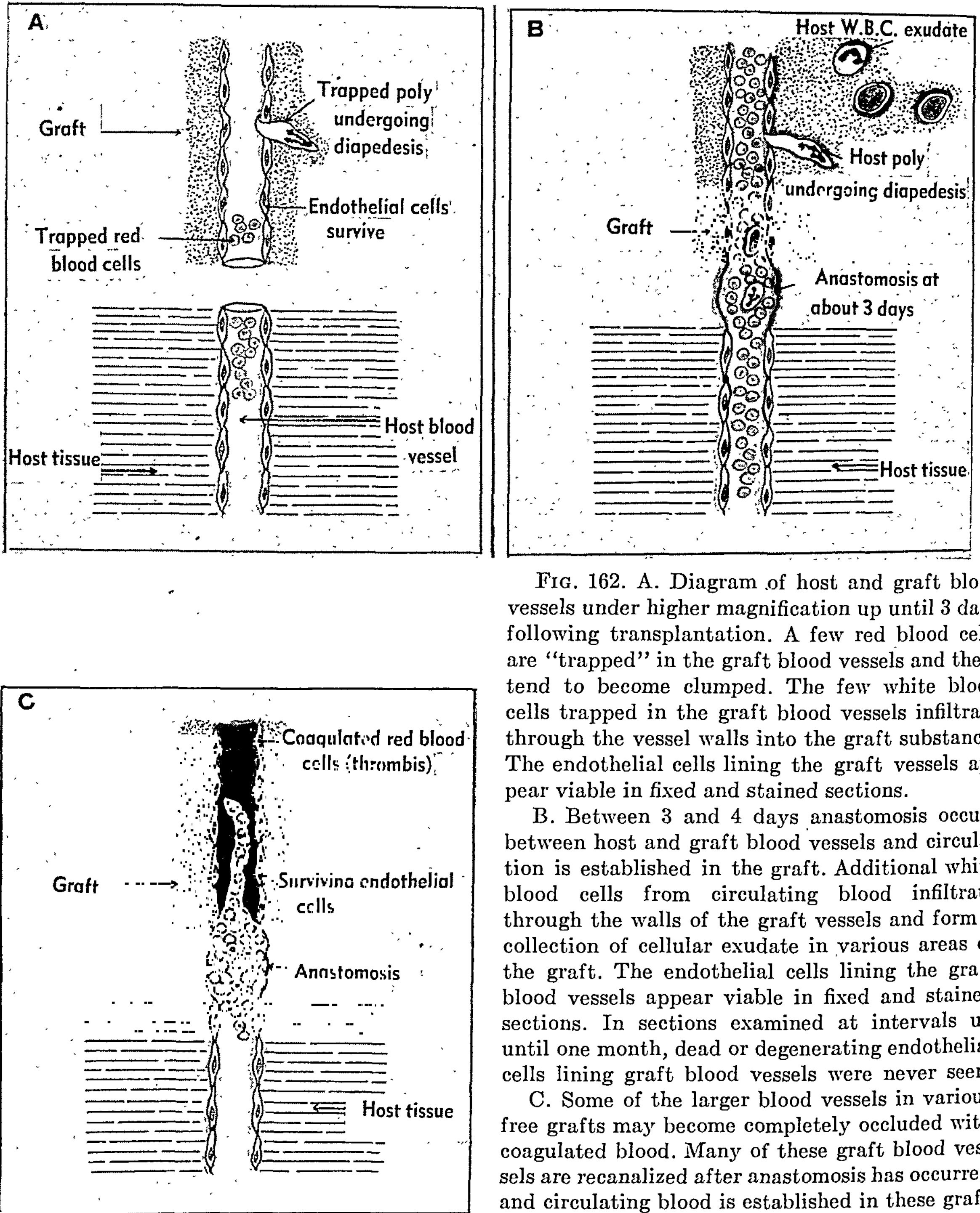


FIG. 162. A. Diagram of host and graft blood vessels under higher magnification up until 3 days following transplantation. A few red blood cells are "trapped" in the graft blood vessels and these tend to become clumped. The few white blood cells trapped in the graft blood vessels infiltrate through the vessel walls into the graft substance. The endothelial cells lining the graft vessels appear viable in fixed and stained sections.

B. Between 3 and 4 days anastomosis occurs between host and graft blood vessels and circulation is established in the graft. Additional white blood cells from circulating blood infiltrate through the walls of the graft vessels and form a collection of cellular exudate in various areas of the graft. The endothelial cells lining the graft blood vessels appear viable in fixed and stained sections. In sections examined at intervals up until one month, dead or degenerating endothelial cells lining graft blood vessels were never seen.

C. Some of the larger blood vessels in various free grafts may become completely occluded with coagulated blood. Many of these graft blood vessels are recanalized after anastomosis has occurred and circulating blood is established in these graft blood vessels. The surviving endothelial cells appear viable in fixed and stained sections. All vascular free autogenous grafts in humans show a similar pattern in regard to their developing circulation.

entire cornea or portions of it may be replaced by infiltrating host cells although work concerning it has not as yet been published. If blood vessels and host fibroblasts invade the cornea, the graft becomes opaque and loses its specific corneal tissue quality of glass-like transparency; thus it is not replaced as the same type of tissue.

Fresh homogenous fat (8) and muscle grafts (3) are completely absorbed and replaced by fibrous tissue. Homogenous surface skin grafts are destroyed grossly in about two weeks, and buried homogenous dermal grafts are replaced by host fibrous tissue.

An accurate description regarding the fate of fresh and preserved homogenous fascia, tendon and nerve grafts is difficult to obtain from the literature. One knows that the cells in fresh homogenous grafts of these tissues fail to survive. Preserved fascia and tendon in contact with like tissues or in abdominal fat appear to be repopulated by living cells, and tendon and fascia grossly retain their general structure for some months after transplantation. In the author's experience grafts up to 4 months showed a rather intense host cellular reaction outside and within the grafts and a large number of engorged blood vessels. Obviously further work involving a study of grafts buried for longer periods of time should be undertaken.

Heterografts

The only heterografts of any clinical use at this time are cartilage and bone grafts, and possibly blood vessel grafts.[7]

Preserved cartilage grafts from the giant sting ray and from the ox cause a more intense cellular reaction than preserved homogenous cartilage grafts, and they are both eventually replaced by fibrous tissue and not by cartilage.

Heterogenous preserved bone grafts in contact with living host bone may be replaced as bone by creeping substitution from the host tissues. The healing is delayed, however, and not infrequently the heterograft becomes extruded.

SCOPE OF TISSUE TRANSPLANTATION

In the present chapter an attempt has been made to present the known and inferred facts regarding the behavior of some of the important tissue grafts together, so that conclusions can be drawn regarding any common action occurring in them. This comparative study may aid in getting rid of older conflicting viewpoints and serve as a basis for future experimental work.

In considering the behavior of free autogenous tissue grafts *one should emphasize their tenacious and usually successful effort to survive and retain the specific tissue structure rather than their occasional failures* (excepting muscle, which always fails).

The statement that certain human tissue grafts survive and retain their specific structure following transplantation, however, is somewhat relative. "Nothing living is actually stable, and life is the continuing effort to maintain equilibrium against forces tending to oppose it." (Cowdry)

Thus, portions of an autogenous cartilage graft may be replaced by bone or fibrous connective tissue, and host blood vessels may grow into cartilage grafts, associated with some absorption. Free bone grafts may undergo a change in size and density due to "physiological turnover," which takes place in all tissues throughout adult life. Fat grafts and surface skin grafts may fail to "take" for no apparent reason.

These occasional variations in behavior do not affect the validity of the cell survival theory. This theory, which is based on statistical studies of large numbers of cells and

[7] Hufnagel (13) implanted frozen-dried blood vessel heterografts into patients when homografts and autografts were not available. This procedure has now been carried out with maintenance of continuity in 8 patients over periods of 3 to 14 months.

dermal grafts, and even free muscle grafts. It is therefore probable that the circulation develops similarly in all free autografts which normally have a vascular system of nourishment (a few exceptions are epidermis, cartilage, lens, and cornea). Moreover, dead or dying endothelial cells were never seen in the graft blood vessels.

In general, our own experimental evidence favors the belief that the vascular system in all free autogenous grafts probably survives and that early circulation is established largely through anastomosis between host and graft blood vessels, and that this remains as the permanent circulation.

Thus, the endothelial cells lining graft blood vessels may conform to "the cell survival theory" rather than the theory of host tissue replacement. As a matter of fact, the survival of the parenchymal cells in thick vascular tissue grafts is largely dependent on the survival and early function of blood vessels in the graft.

It is usually stated that free grafts are nourished by the host tissue fluid until a vascular supply has been provided, and this is probably important for cells near the surface of the transplants; at any rate it prevents desiccation, which will kill any cell. Most cells which are kept in their normal fluid environment at body temperature do not die rapidly from accumulated waste products or lack of oxygen (with the exception of skeletal muscle cells). Naturally cells will survive longer when their metabolic processes are slowed down by lowered temperature.

BEHAVIOR OF HOMOGENOUS AND HETEROGENOUS TISSUE GRAFTS

Homografts

In human homografts the story is somewhat different since unprotected homogenous cells fail to survive for long periods of time in a hostile environment.

Homogenous bone grafts, both living and dead, tend to be gradually absorbed and replaced by new bone from the host tissues if the graft is in contact with bone. If the homogenous bone graft is in contact with soft tissues, the graft structure is absorbed and replaced by fibrous tissue. The osteocytes and other cells in the fresh homograft of course die in a short time after transplantation.

Homogenous cartilage grafts, both fresh and preserved, frequently retain their cartilaginous structure for long periods of time. They are, however, slowly but progressively invaded and replaced by fibrous tissue and sometimes by bone *but they are not replaced by cartilage.*

Fresh homogenous corneal grafts lose their external epidermal covering and their internal covering of endothelial cells; both of these losses are replaced by host epithelium and host endothelium respectively, which recover the thick collagenous zone on both of its exposed surfaces. This thick collagenous zone appears to remain, and the sparsely scattered fibroblast or fibrocyte cells in the zone remain viable due to the protective action of the avascular collagenous matrix. The matrix of the cornea is similar to the matrix of cartilage, and it is interesting to note that both the corneal fibrocyte cell and the cartilage cell appear to remain viable as long as they are protected by their avascular intercellular substances. When this substance is removed and the cells are exposed to more direct exchange from penetrating blood vessels, the cells disappear. Under favorable conditions many fresh homogenous corneal grafts remain clear, and it is generally accepted that the cells remain viable and retain their characteristic clear collagenous matrix. The success of the fresh homogenous corneal graft has served as a bulwark to encourage experimental work with other fresh homogenous tissue grafts. There are, however, some experimenters and clinicians who believe that the

plished in the body with bone (os novum) but this has limited clinical application. Attempts to grow tissues in artificial media and later to graft the transplants back in the same individual as autografts have not been thoroughly investigated; this approach has potential possibilities. In theory such a graft would be biologically compatible with its original donor and later recipient provided undesirable changes did not occur during the *in vitro* growth period.

It seems evident that the medicine and surgery of the future will be based on a more accurate knowledge of that minute biological unit, the tissue cell, and its collective structures, the tissues and organs. The physician and surgeon should therefore bring himself into closer relationship with the laboratory worker and become thoroughly familiar with the results of experimental data which may be applied for clinical use in man.

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their adjacent matrix, *describes the usual behavior of the majority of cells in the various tissue grafts.*

The question properly arises: Why all this bother about establishing a law or a theory governing the behavior of free autogenous tissue grafts?

This can be answered in the following way. When an individual studies the behavior of more than several substances, animals, or tissues he inevitably must speculate about what has been seen. In a study of the behavior of tissue grafts there is real value in establishing and stating a rule that describes the usual or average behavior which all, or nearly all, possess in common. Although many of the factual findings in this book may be accurate, about 50 per cent of the interpretations and conclusions will probably be wrong. These erroneous conclusions will be exposed by future investigators and our knowledge regarding the behavior of free tissue grafts advanced.

Certainly the striking thing about the cells in human tissue grafts is their ability to survive the shock of severed blood, nerve and lymph supply and reestablish all of these when the host environment is favorable, which is to say, is one which is similar to their natural habitat.

Experimenters in the fields of pure scienct have added greatly to our knowledge of cell structure and intricate cell metabolism during recent years.

It is interesting to note that microdissection and microtransplantation have entered the field of grafting. Dissection of cells under the microscope by manipulating instruments and the transplantation of various structures from cell to cell have possibilities of tremendous magnitude.

Single tissue cells in a hanging drop have been transferred from one animal to another, and adult human tissues obtained from the operating room are grown in tissue culture. Possibly small fragments of epidermis taken from a severely burned patient can be grown into large sheets in tissue culture. These may be used later to cover extensive raw areas on the body surface of the same patient.

There is evidence that fetal tissues, which have not developed a sensitivity to antibodies or the capacity to cause their production, can be successfully transplanted as fresh homografts.

Fresh homograft kidneys have been transplanted to a patient's thigh, the renal vessels being joined to the femoral vessels. Nine of these "blood transfusion" transplants have excreted urine through their ureters on the external surface of the thigh for periods as long as 90 days. The problem of successfully transplanting other organs and hormonal glands may be about the same as that of transplanting homogenous kidneys.

All of the foregoing will be dealt with at length in Volume II, with a description of the behavior of other free tissue transplants such as skin, fat, nerve, teeth, mucous membrane, cornea and lens, and the cancer cell.

Experimental studies regarding the fate of homografts are important basically for a better understanding of cell behavior and require no justification from the standpoint of clinical application. It follows naturally, however, that the clinician should profit by the investigations of pure scientists and apply the results of these findings for practical uses in humans. Actually the main reason for the interest of the medical man in homografting is because of the fact that the supply of autograft material is not available (endocrine glands, organs), is limited (skin, mucous membrane) or not practicable to use (cornea).

There are two apparent avenues of approach in solving the problem of absent or poorly functioning tissues (aside from injecting hormones etc.): one is to transplant homografts successfully and the other is to increase the supply of autograft material. The latter has been successfully accom-

Index of Names

Index of Subjects

living bone, ivory is gradually absorbed; it does not loosen, nor does it produce necrosis or softening in bone immediately in contact with it. He considered it advantageous to use ivory screws in oblique fracture; they are gradually absorbed and do not act like foreign bodies.

Gallie and Robertson (7) (1919) reported on the bridging of gaps in 5 patients with Pott's disease, utilizing boiled human bone, beef bone, dog bone and in one case a piece cut from an old skeleton. Three years after these operations four of the patients were still alive and as well as if they had been treated by any other method. In Gallie and Robertson's series of 100 operations in which boiled plates and screws of beef bone were used in fractures, no plates ever had to be removed. They found that when heterogenous or boiled bone is placed in close contact with living bone, the resulting gross and histological changes closely resemble those which follow the implanting of autogenous bone.

Ryerson (8) (1919) maintained that in fresh fractures the use of beef bone was satisfactory although autogenous grafts were preferable in non-union and in fractures in elderly persons.

Henderson (9) (1920) observed that beef bone screws were well tolerated by the tissues and absorbable, and advocated their use in place of metallic screws. Up to the time of the introduction of vitallium, beef screws were used at the Mayo Clinic for many surgical purposes.

The use of dead boiled beef bone splints preserved in alcohol was advocated by Brenizer (10) (1920) for patients with osteomyelitis—not, however, when the disease is a primary consideration.

Neuhof (11) referred to an instance of implantation of the femur from a rabbit between the free fragments of an injured femur in a human. The functional result was excellent and was corroborated by roentgenograms 6 months later. Cunningham (12) (1924) agreed with this opinion. Beef bone is simpler to prepare and use than autogenous grafts and is preferable to metallic plates.

In 21 cases in which beef bone splints were used, union was perfect in 18, as reported by Harris (13) (1925). The splints were absorbed in 18 to 24 months. Union was delayed in 2 patients. Kleinberg (14) (1926) reported 33 cases of previously-prepared beef-bone grafts for fusion in scoliosis; in only one patient was there sufficient irritation to cause drainage. In 1929 he reported 58 successful cases of spinal fusions by means of grafts of beef bone.

Bailey (15) (1927) used beef bone cleaned, cut to pattern and boiled for 5 to 6 hours, in two patients. In one patient the graft fitted into a bed prepared by splitting the spinous processes. After 2 months there was a steady return of function, sphincter control, sensation, and motor power; the patient walked in 8 months. In the other case, after ox bone had been inserted, the patient walked quite well a year later and was free from symptoms.

Tavernier (16) (1930), basing his opinion on his experience with grafts of heterogenous bone preserved in alcohol, expressed the belief that it is a surgical material without great value and inferior to pieces of metal or ebony, which are not less tolerated. His cases showed that even under the most favorable conditions, as in interspinous graft, the dead heterogenous bone did not unite with the tissues with which it was in contact and was resorbed in time. An osseous piece showed zones of resorption, not interstitial and haversian as in experimental pieces but without substitutive building of new bone; he compared these observations with Nageotte's on minute osseous fragments buried under the ear skin of a rabbit as being under rather different conditions from those of large surgical grafts.

Employing cow's horn, Fowler (1934) noted that nine fractures united promptly with superabundance of callus and bony union, without complications. The horn was gradually absorbed (17). In search for an explanation of the apparent stimulating effect of callus formation, he reviewed the literature on the subject. In 30 cases including his own where horn had been used for internal fixation of fractures, it seemed to have been ideal material for strength, elasticity, absorbability and ease of sterilization. It appeared to be non-irritating, mildly bactericidal and stimulating to callus growth (18).

Calvé (19) (1935) employed heterogenous spongiose tissue from a young calf for vertebral osteosyntheses, tuberculous arthritis, and arthritis in 15 operations. The osseous substance was admirably tolerated, and the clinical results were excellent. The fragments of calf tissue in small bags were preserved in ether. At the time of use the fragments were bathed carefully in artificial sterilized serum. In a case of pseudarthrosis of the tibia grafted with ox bone Leriche (20) (1935) confirmed the complete cure 9 years after operation. He considered the permeable factor of bone most important.

The use of heteroplastic grafts was fully justified in a number of cases of pseudarthrosis and also in recent fractures in which the use of metal was inconvenient and difficult, as reported by Danis (21) (1936). From actual experience he was favorably impressed with the use of this grafting material. When the wound remains aseptic, the ox bone is very acceptable. But infection makes it untolerated more quickly than when fresh human bone is used. He was not able to judge the late results of his heteroplastic transplants. He raised the question whether os purum will give proof of revitalization (modifying form and intimate structure) as has been observed in autoplasties, which always respond to the mechanical needs of the skeletal environment.

Carrell (22) (1936) advocated the use of prepared cow's horn as material for internal fixation in bone surgery and used it in 40 cases. He believed it has adequate strength and produces abundant callus formation without reaction.

Orell's os purum and os novum have been previously discussed under preserved homogenous bone grafts, for he used both ox bone and human cadaver bone prepared by a definite process before being finally washed, dried and sterilized by boiling. He used these materials with considerable success. A number of other surgeons, incited by his favorable report, undertook further experiments to prove the worth of these grafting materials (23).

For mechanical fixation Zygmunt (24) (1937) advocated the employment of heterotransplants which, he believed, were resorbed and transformed like autoplastic material. The implantation of a graft induces hyperemia, which accelerates the separation of diseased tissue and stimulates the osteogenic proliferation. Beef bone and bird bone were used for osteosyntheses, shelf operations, fractures, pseudarthroses and other conditions.

In a case of a boy of 18, reported by Groves, ivory from a walrus tusk was used to fill a large cavity in the femur in 1922. In 1938 the ivory had not undergone any change. In a boy of 10, with fibrocystic disease of the right humerus, beef bone was used to reconstruct the humerus. The length and general structure were the same as those of the unaffected humerus. In this boy, who became an athlete, ghostly shadows of beef bone could be traced in the midst of human tissues 10 years later. In a woman with a fracture of the neck of the femur, Groves constructed a bone peg made from a stag's antler. She could walk well a year later. In radiographs taken 3 years later the bone

peg appeared to be completely absorbed and replaced by human bone (25).

At Esnaurrizar's clinic in Mexico City, in 1940, a heterogenous os purum graft was placed deep in split spinous processes in tuberculosis spondylitis, with an entirely satisfactory result, and excellent tolerance. In another patient an os purum graft was transplanted in pseudarthrosis, with a good result. In a third case a "fresh" bone graft obtained from an abattoir was used for old fractures of the patella, the result proving to be excellent (26).

Nyst (27) (1941) held that os purum, or purified beef bone, can replace autogenous grafts in many cases. He discussed its advantages and disadvantages. The insertion of beef bone in fractures with delayed callus formation by a special method, which is described in detail, makes the process take a favorable course. It is also a suitable material for osteosynthesis.

Van der Noff (28) (1941) stated that os purum had twenty times the elasticity of steel and was plastic. He inserted two small pieces across the fracture line for fixation at right angle to one another. In 1944 he asserted that the advantages claimed for os purum were not borne out by careful scrutiny. Beef bone was as satisfactory though it required longer to become completely absorbed.

Ghormley and his colleagues have used beef bone screws for years to fix grafts. In 1939 he reported on the use of heterogenous eye teeth of walruses, stags' antlers, and beef bone as grafting material. He believed that circumstances must be extraordinary to justify the use of heterogenous bone (29).

Ghormley (1942) expressed the view that dead bone may ultimately become revascularized and replaced by new bone, as observed in the disappearance of beef bone screws in grafts. At times the continued presence of bone in radiographs may be an indication of "retarded bone metabolism" (29).

In his judgment, the heterogenous grafting method seems to have gained some popularity, particularly in Europe.

Judet and Arviset (30) (1949) prefer the large epiphysis of the calf with its abundant spongiose content and its thick cortical bone. The femur of the animal is frozen at −25°C. for 48 hours and then kept at −15°C. They applied numerous grafts in massive parts or small pieces in most varied cases: spinal grafts, pseudarthroses, fresh fractures, and osseous cysts. There was one postoperative incident of drainage of abundant serum for two months. In other cases cicatrization by primary intention and immediate normal evolution took place. The appearance after these heterogenous transplantations was the same as following homografts, without the process of resorption. The humerus consolidated in 70 days. Judet and Arviset concluded that animal heterograft after freezing at −15°C. can be used in humans without phenomena of intolerance. Animal transplants have value and act in the rôle of a graft when after delay of six months they have not shown resorption. The consolidation in pseudarthrosis testifies to the "vitality" of the graft.

Leriche (31) (1950) reported the interesting case of an officer who had already been operated on three times for pseudarthrosis of the tibia, the operations having consisted of sliding osteoplasty in May 1917, transplantation of a fibula in November 1917, and contraction of both bones in June 1918. He then made a transplantation of the Albee type on February 4, 1920. The results were good but a fracture of the graft occurred and pseudarthrosis returned. After freshening both bones Leriche fastened the two fragments of the tibia together with a peg of ox. bone, thrusting it down to firm contact on April 18, 1920. The union was solid in two months. A radiograph at 15 months postoperatively showed the peg in place, with the formation of new bone at the periphery. The tibia was

a solid block larger than formerly. Twenty years later (1941) word was received that the officer was walking normally and radiographically had a large dense tibia, which was not painful. It appears to Leriche that the ox bone peg is playing an osteogenic rôle.

Following his successful experience in 1920, he made four transplantations of dead bone, one of which was human bone preserved in alcohol, for loss of substance in the ulna, with excellent results two years later.

Stagnara and Dubost-Perret (32) experimented with calf bone, treated four times and preserved in refrigeration at −35 and then −70°C. until utilization. Anatomically, radiographically and histologically they studied the fate of transplants of different types: spongiose and compact bone, in autografts and heterografts, fresh and refrigerated grafts; and as onlay and insertion into osseous section. Their clinical experience confirmed the findings in the laboratory perfectly. In 16 surgical interventions in which refrigerated calf bone was used on 14 patients with tumors of the tibia, bony cysts, pseudarthrosis of the femur and so on, the grafts were tolerated without difficulty.

Having obtained encouraging results from their animal experiments (Chapter 16, page 172) Guilleminet, Stagnara and Dubost-Perret (33) (1950) used sheep bone in humans for cavities, surgical ankyloses of diverse articulations or chronic arthrites, deformations or infections, and for pseudarthroses.

The successful animal experiments on heterogenous osseous grafts carried out at the Clinique Chirurgicale Orthopédique et Infantile de Lyon (Guilleminet in charge) encouraged the utilization of prepared animal bone in human surgery. During the last three years calf bone has been very generally employed in conditions indicating the application of bone grafts. The last chapter of the film described (see also Chapter 16) shows three such surgical interventions (34).

All heterogenous bone grafts used by the Judets (35) (who established the first French bone bank) were frozen at −25 to −30°C. and immediately afterward placed in the deep freeze. In 77 cases in which osseous heterografts were employed, there was no aseptic elimination of any of them. Perfect tolerance of frozen heterotransplants appears to be a well-established fact. Two indisputable signs of life of a graft are its increase in volume, and the consolidation of a fracture with the graft. Success was recorded in 24 per cent of the operations, failure in 32 per cent, and good results clinically in 23.37 per cent—but difficult to interpret radiographically. Grafts of bone from the pig, calf or colt seemed to evolve in the same manner. The Judets considered the colt the best donor animal. The degree of refrigeration is of little importance. The results were analogous to those from homografts refrigerated at −10°C. The Judets found that grafts in a bridge or in a point are resorbed, while those amply and closely applied to the osseous bed take and fuse. This appears to them to be a fundamental law.

In their opinion, heterografts furnish, in unlimited quantity, a material of equal value as bone homografts taken at operation or from cadavers.

SUMMARY COMMENT ON HETEROGENOUS BONE GRAFTS

The published evidence indicates that heterografts may induce the process of osteogenesis after transplantation in the human. Presumably the graft structure under favorable conditions may be slowly absorbed and replaced in kind as osseous tissue by the host bone, the host periosteum or the host connective tissue. Bone heterografts as screws and plates appear to be more popular abroad than in the United States, where metal is more commonly used for this purpose.

I have had no experience with bone heterografts and can only draw conclusions from published reports regarding their use. These reports vary with different investigators.

Quite probably beef bone is about as valuable as ox cartilage, which Armour and Company sell at a rather high price. Ox cartilage is inferior to homogenous cartilage, which in turn is inferior to autogenous cartilage as a grafting material. Heterogenous bone grafts are a distinct third choice in the field of bone grafting. Considering the availability of frozen and preserved homogenous bone grafts when autogenous bone may not be used, there is little if any reason to use animal grafts in the human. Fresh homogenous bone grafts are probably as dependable as the frozen variety and their behavior is the same.

In general, foreign grafts are believed to stimulate an immune response in the host tissue, and this immune response is detrimental to the clinical success of various tissue grafts in various degrees, as demonstrated by Medawar (36).

Eichwald (37) has commented that there exists a paradox of too much immunity when it is not wanted and too little immunity when it is wanted. The foreign graft evokes an immune response which defeats us. By contrast, when we develop cancer, it is our own cancer and it does not tend to create the immune reaction which would destroy the growth.

The long bones, the spinal column, and many other human bones, however, have the ability to replace dead bone grafts with new living bone, and this appears to occur even when heterogenous bone grafts are used.

Os purum is a specially prepared hetero- or homograft, depending on whether human or animal bone is used. Os novum represents newly-formed bone from the host tissues which has replaced the os purum, and it is an autogenous bone graft. The physiological principles in its formation are very interesting but its clinical use necessitates two separate operations. If healing is more rapid, this type of graft may be indicated in selected cases; otherwise a direct autogenous bone graft in a single operation would be preferred.

Drawings Indicating Usual Behavior of Bone Grafts in Man

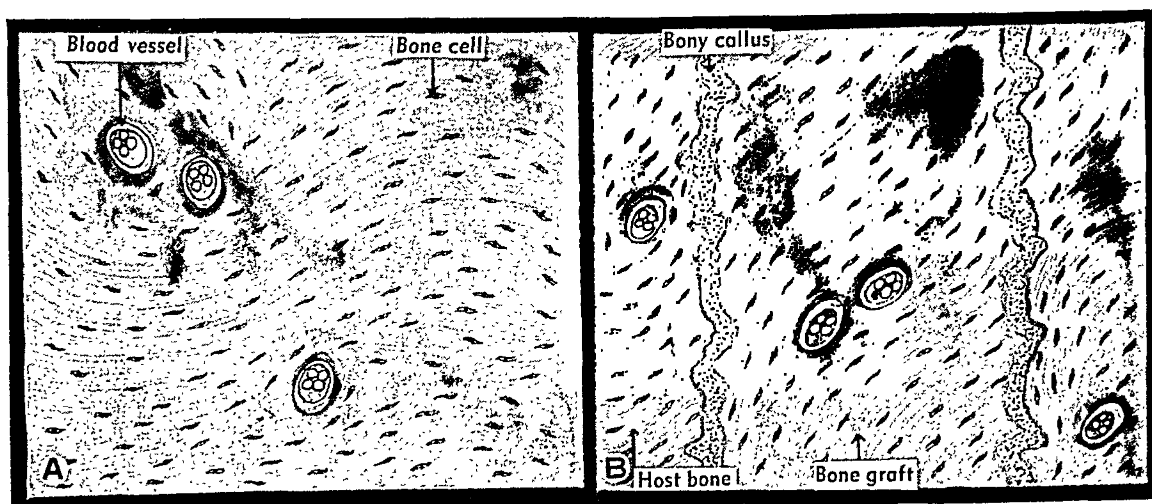


FIG. 77. A. Fresh autogenous human rib, tibial and iliac bone of the cancellous variety, both with and without periosteum, transplanted in contact with bone.

B. Many of the bone cells (osteocytes) probably survive. The bone graft is joined to the host bone by callous formation as in healing fractures.

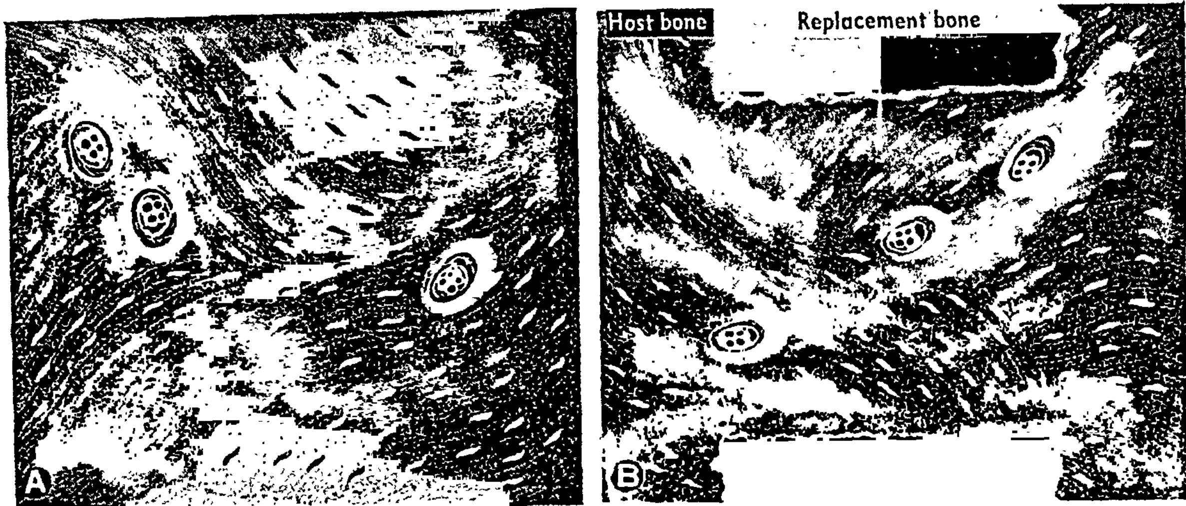


Fig. 78. A. Fresh autogenous human dense cortical bone from tibia, with or without periosteum, in contact with bone.

B. All or most of the bone cells in the graft die and the graft is replaced by creeping substitution.

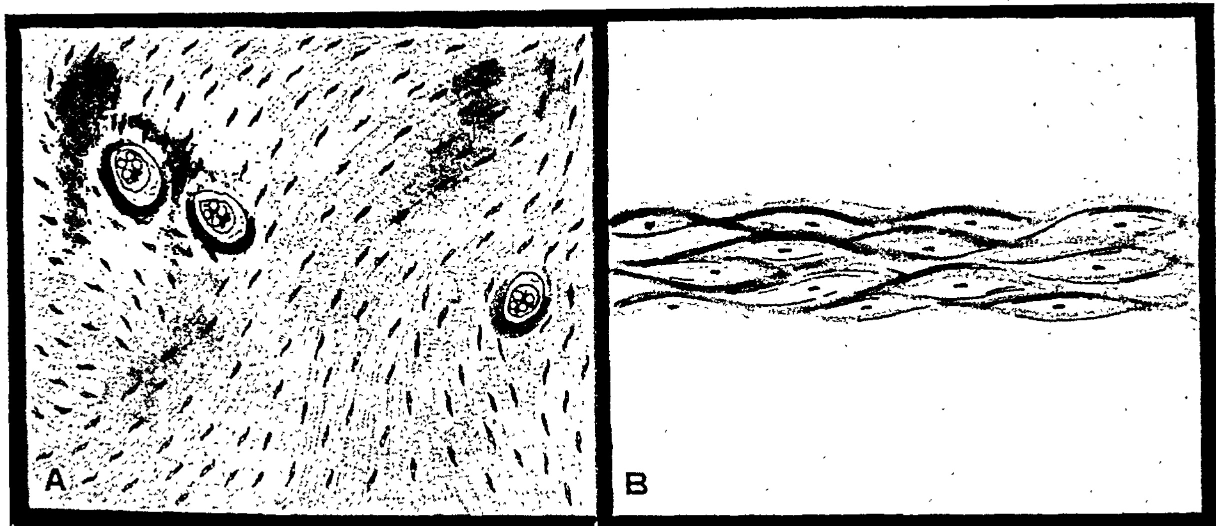


Fig. 79. A. Fresh autogenous human rib, tibial and iliac bone, either cancellous or cortical, with or without periosteum, in contact with soft tissue.

B. The grafts are replaced by fibrous tissue.

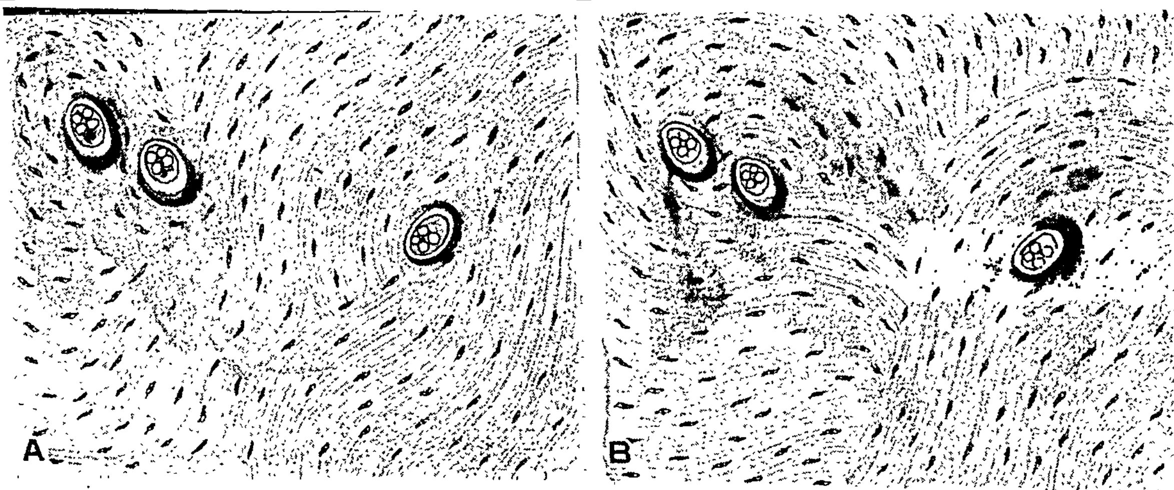


Fig. 80. A. Fresh autogenous human septal, nasal and turbinate bone grafts without periosteum, in contact with soft tissue.

I have had no experience with bone heterografts and can only draw conclusions from published reports regarding their use. These reports vary with different investigators.

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Drawings Indicating Usual Behavior of Bone Grafts in Man

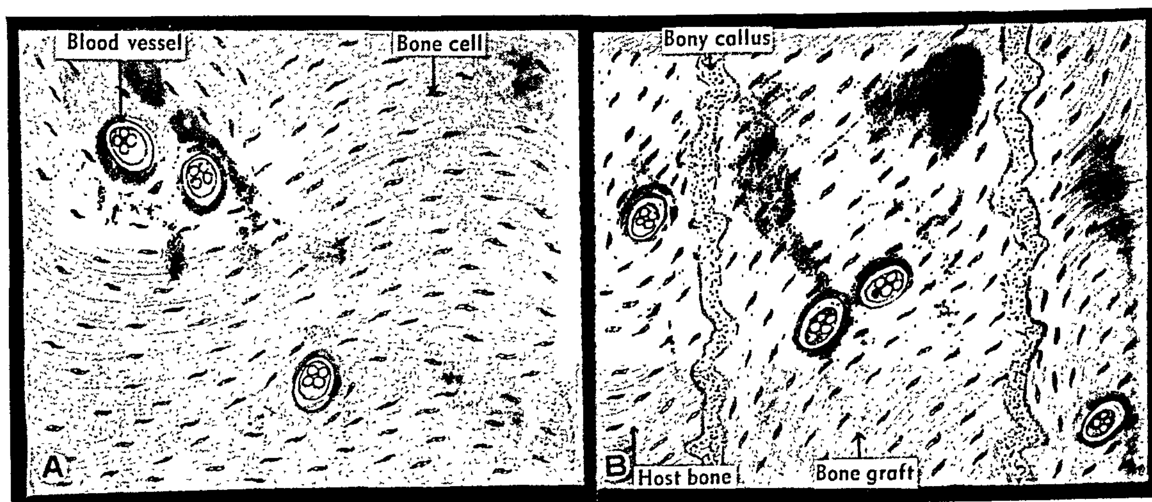


Fig. 77. A. Fresh autogenous human rib, tibial and iliac bone of the cancellous variety, both with and without periosteum, transplanted in contact with bone.

B. Many of the bone cells (osteocytes) probably survive. The bone graft is joined to the host bone by callous formation as in healing fractures.

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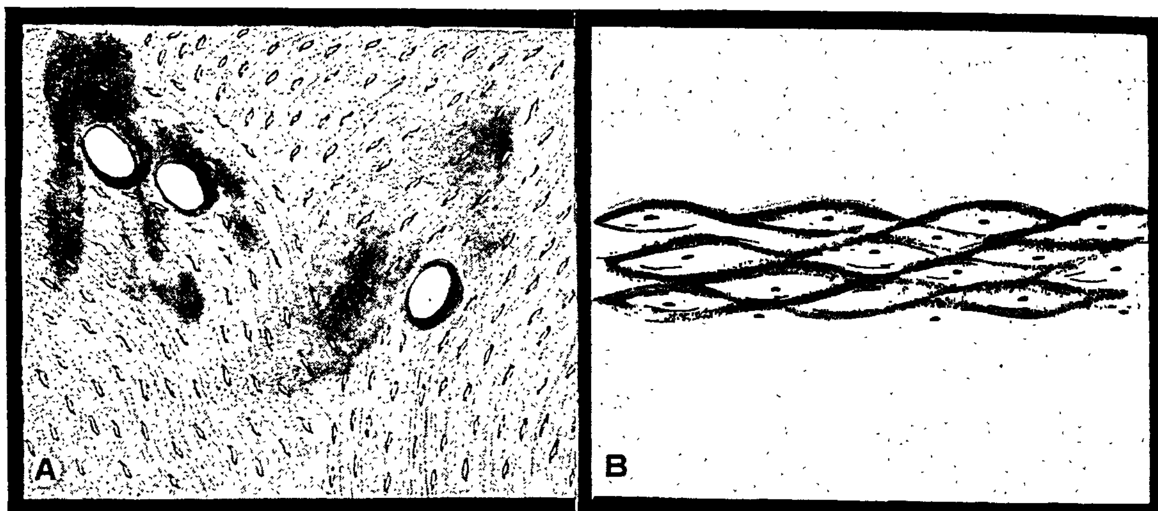


FIG. 81. A. Heat-treated autogenous human rib, iliac and septal bone grafts with dead cells in contact with soft tissue.
B. The grafts are replaced by fibrous tissue.

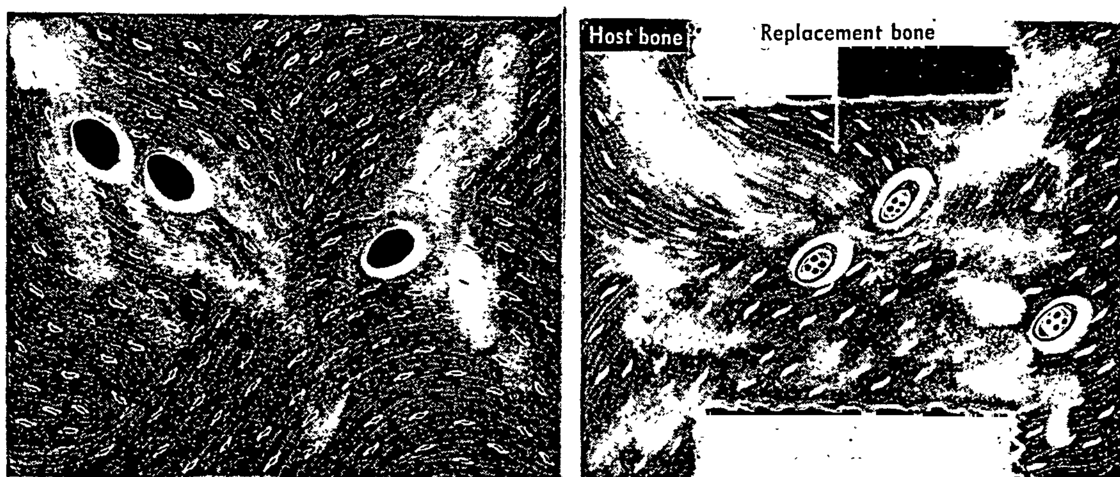


FIG. 82. A. Preserved or fresh homogenous human bone, rib, iliac and tibial, in contact with bone.
B. The graft is replaced by creeping substitution from the host tissue.

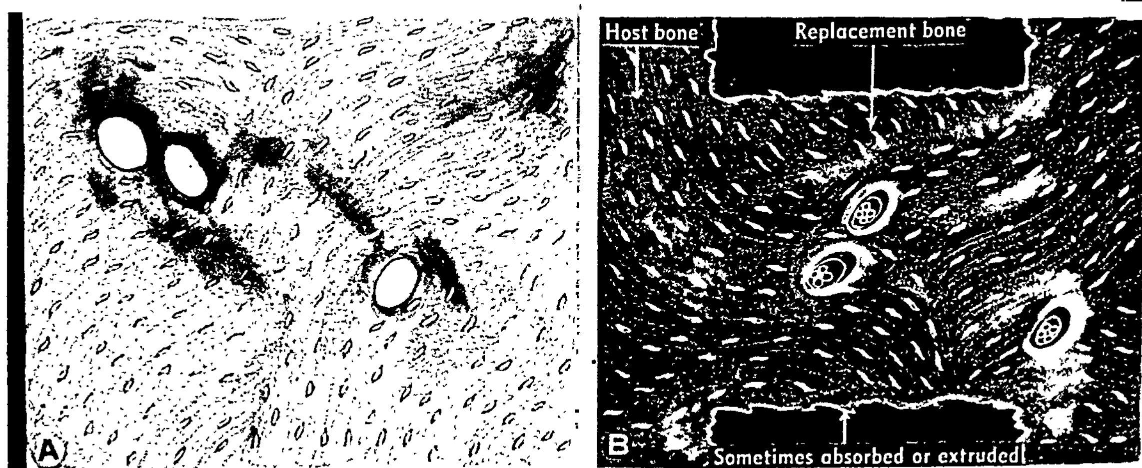


FIG. 83. A. Preserved and heat-treated bone grafts from animals (heterogenous) in contact with bone.
B. These foreign grafts are said to be replaced as bone by creeping substitution from the host tissues. Sometimes the graft is absorbed or extruded.

CHAPTER 20

Clinical Use of Bone Grafts

A questionnaire was sent by the author to a selected group of orthopedists, plastic surgeons and neurosurgeons, asking for an opinion regarding the merits of the different types of bone grafts for clinical use.

QUESTIONNAIRE OPINIONS ON TYPES OF BONE GRAFTS

All of these surgeons *preferred living autogenous bone* instead of homogenous or heterogenous bone whenever the first type was available. Most authorities agreed that homogenous bone, either living or dead, was often replaced by creeping substitution from the living host bone or its periosteum. A few did not use homogenous bone in any circumstance, and none used heterogenous bone grafts. All agreed that the presence of periosteum on autogenous grafts is not necessary for survival of the graft structure, although Sterling Bunnell, Kazanjian and Henry Kessler believe that it is helpful.

Robert Ivy does not think that homogenous bone grafts survive transplantation, but it is possible that they stimulate the living bone with which they are in contact to produce new bone. On the other hand, he is sure that living autogenous bone transplants do survive as such. Ivy further states that he has yet to see a report of a successful homogenous bone graft restoring continuity of a movable long bone in the human. "Bone banks" may be useful as a source of material for filling bone cavities but, he believes, there is a definite limit to their usefulness. Kazanjian feels that the supply of autogenous bone is so abundant and the results so dependable that there is little need to use more undependable materials.

George M. Wyburn and his colleagues in plastic surgery at the University of Glasgow favor the use of autogenous bone grafts. The ilium is preferred as a donor site in most instances. Regarding the results of experimental work Wyburn considers it very doubtful that the presence of periosteum favors the survival of autogenous bone grafts.

Rainsford Mowlem uses only autogenous bone grafts and he believes that they tend to survive and grow under favorable circumstances. He emphasizes that bone is a tissue which is designed to withstand stress and strain. If it is grafted in an area where these factors are not present, its initial survival may be satisfactory; but like the excess callus formed around a fracture it is, unless subjected to stress and strain, ultimately removed and its removal is an expression of perfectly normal body function.

Frank E. Stinchfield also doubts that the periosteum adds or detracts from the usefulness of a graft. If the graft is autogenous and the cells expected to remain permanently